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**Patton et al.**

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(54) **METHOD FOR INHIBITING MICROBIAL GROWTH IN LIQUID NUTRIENTS**

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(22) Filed: **Nov. 10, 2004**

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**Related U.S. Application Data**

(63) Continuation-in-part of application No. 10/936,929, filed on Sep. 9, 2004, which is a continuation-in-part of application No. 10/823,446, filed on Apr. 13, 2004, now Pat. No. 7,258,786.

(51) **Int. Cl.**

**C02F 1/50** (2006.01)  
**B01D 37/00** (2006.01)  
**A23L 2/72** (2006.01)

(52) **U.S. Cl.** ..... **210/764**; 210/638; 422/28; 426/271; 426/330; 426/330.3

(58) **Field of Classification Search** ..... 210/764  
See application file for complete search history.

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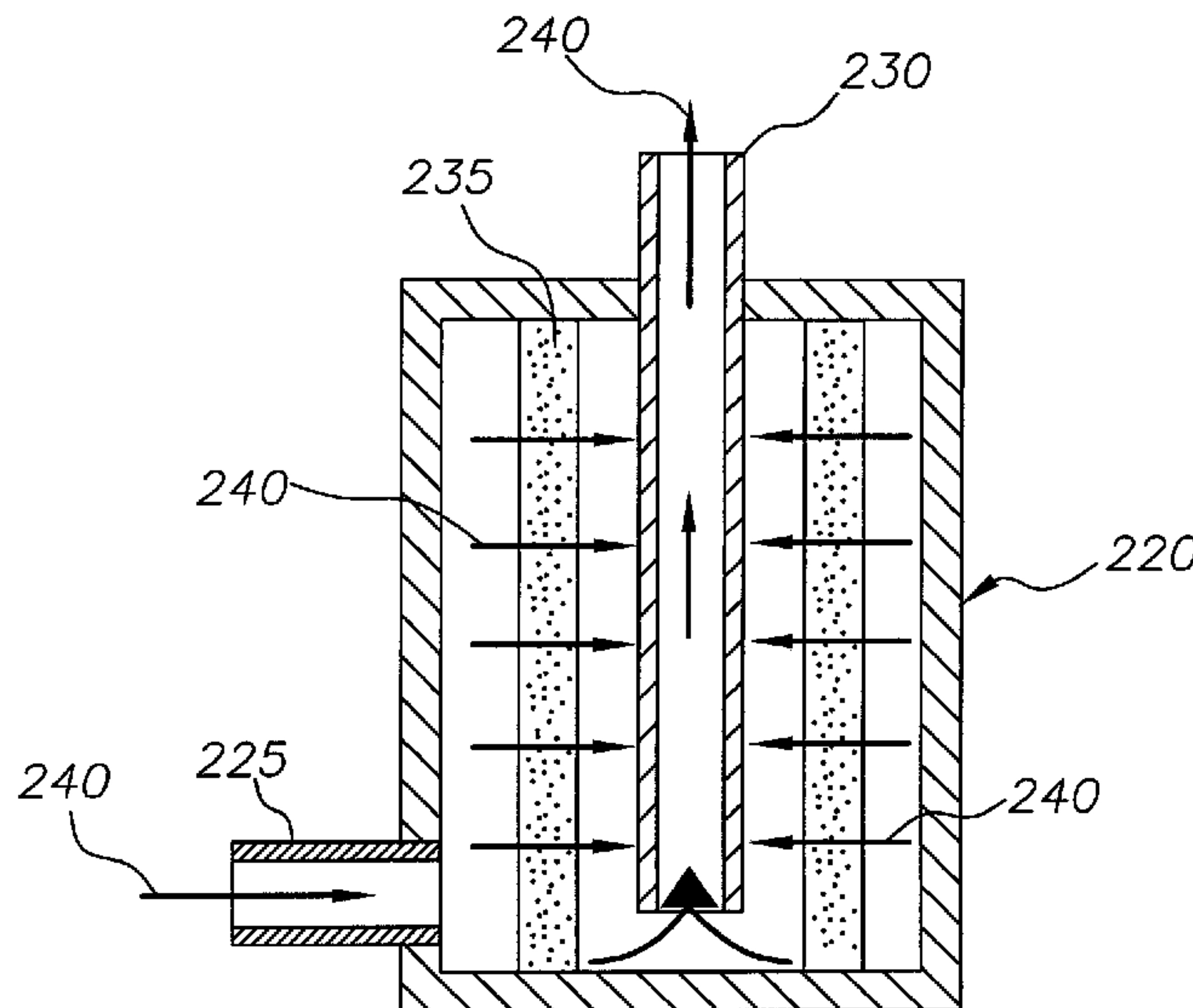
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(57) **ABSTRACT**

A method for inhibiting growth of microbes in a liquid having a pH equal to or greater than about 2.5 by using a filter bed assembly or filter for filtering the liquid having a metal-ion sequestering agent for removing designated metal ion from the liquid. The liquid is passed through the filter bed or filter for a sufficient time so as to substantially reduce the designated metal ion from the liquid.

**44 Claims, 23 Drawing Sheets**



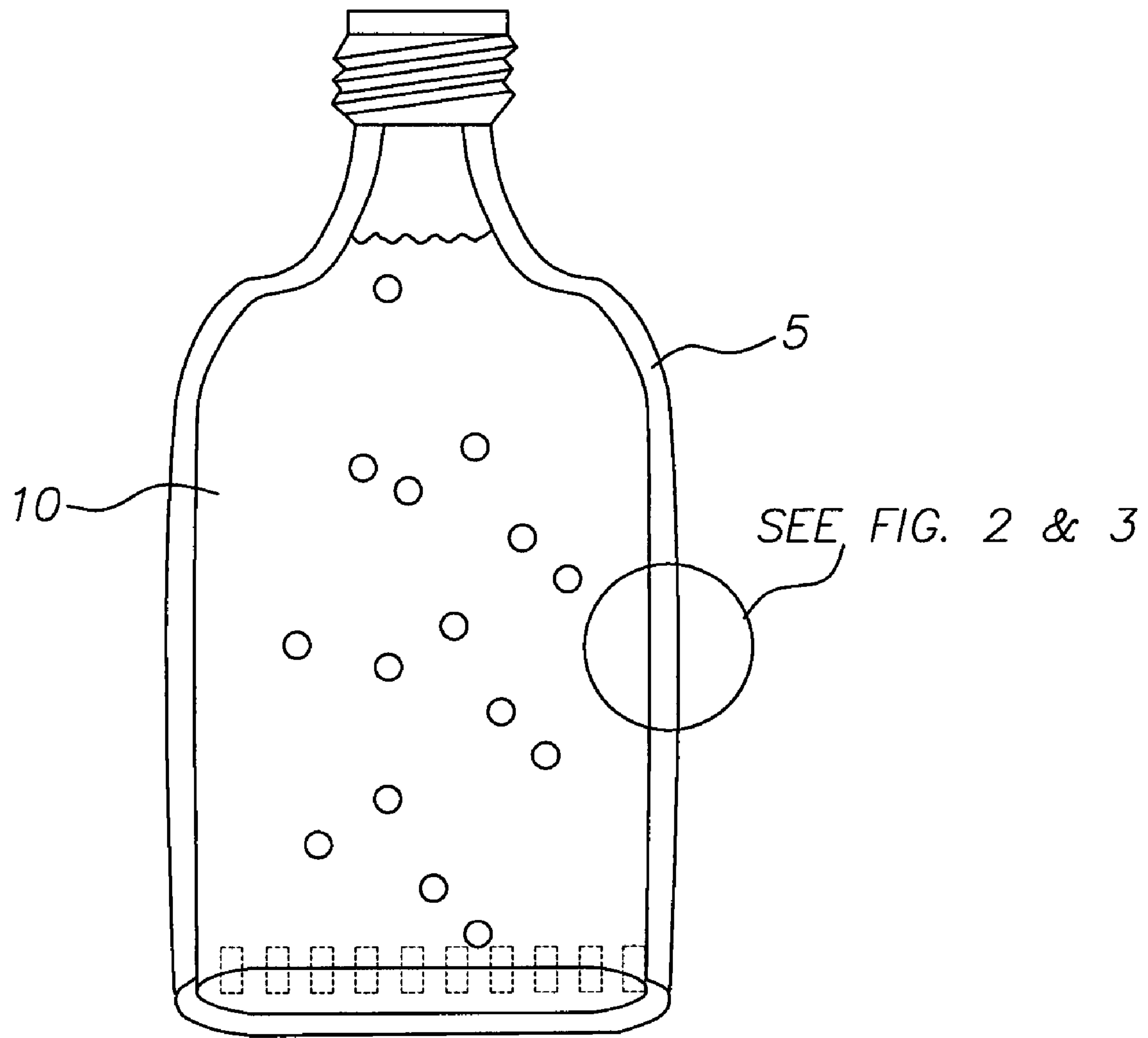


FIG. 1  
(Prior Art)

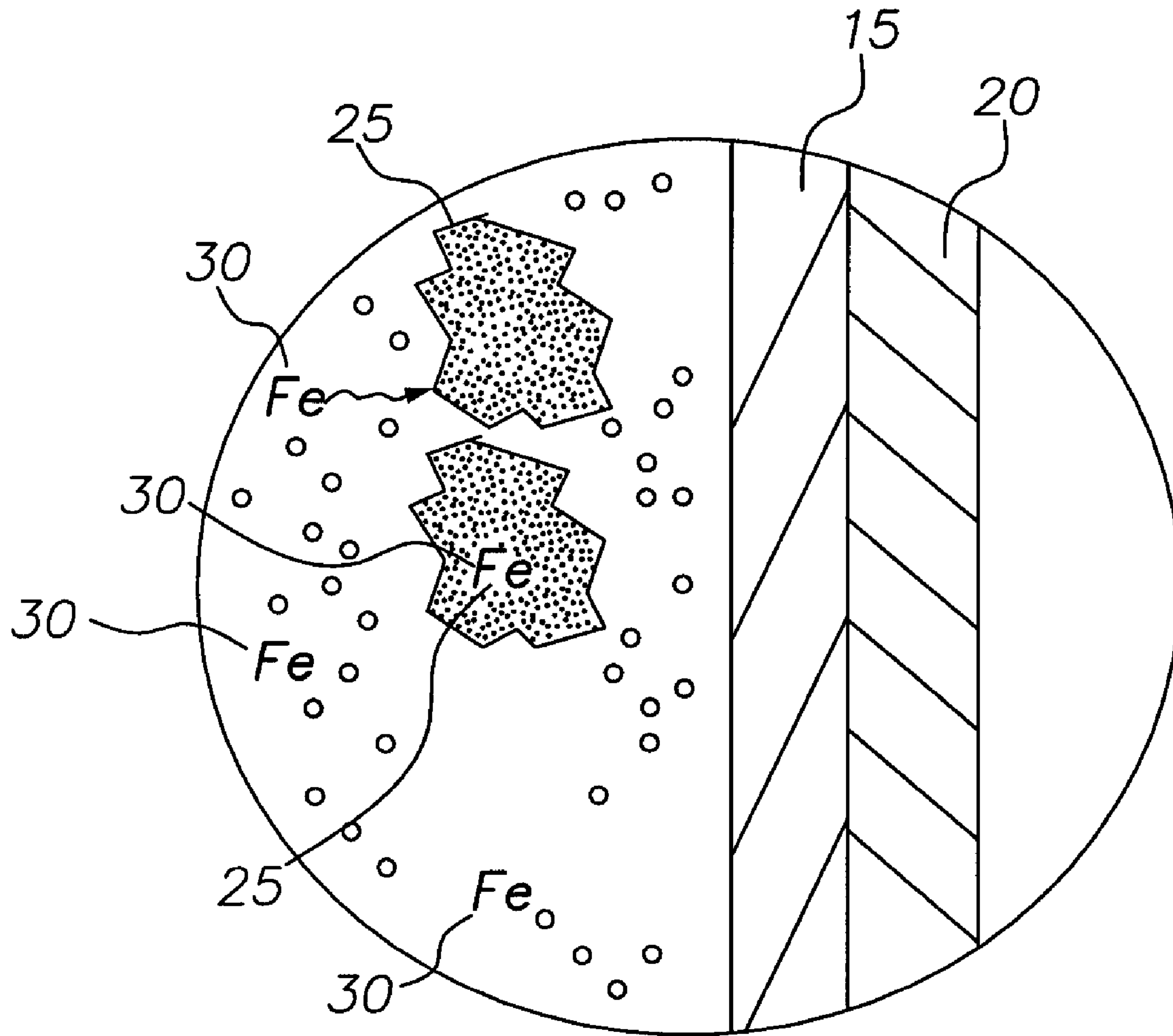


FIG. 2  
(Prior Art)

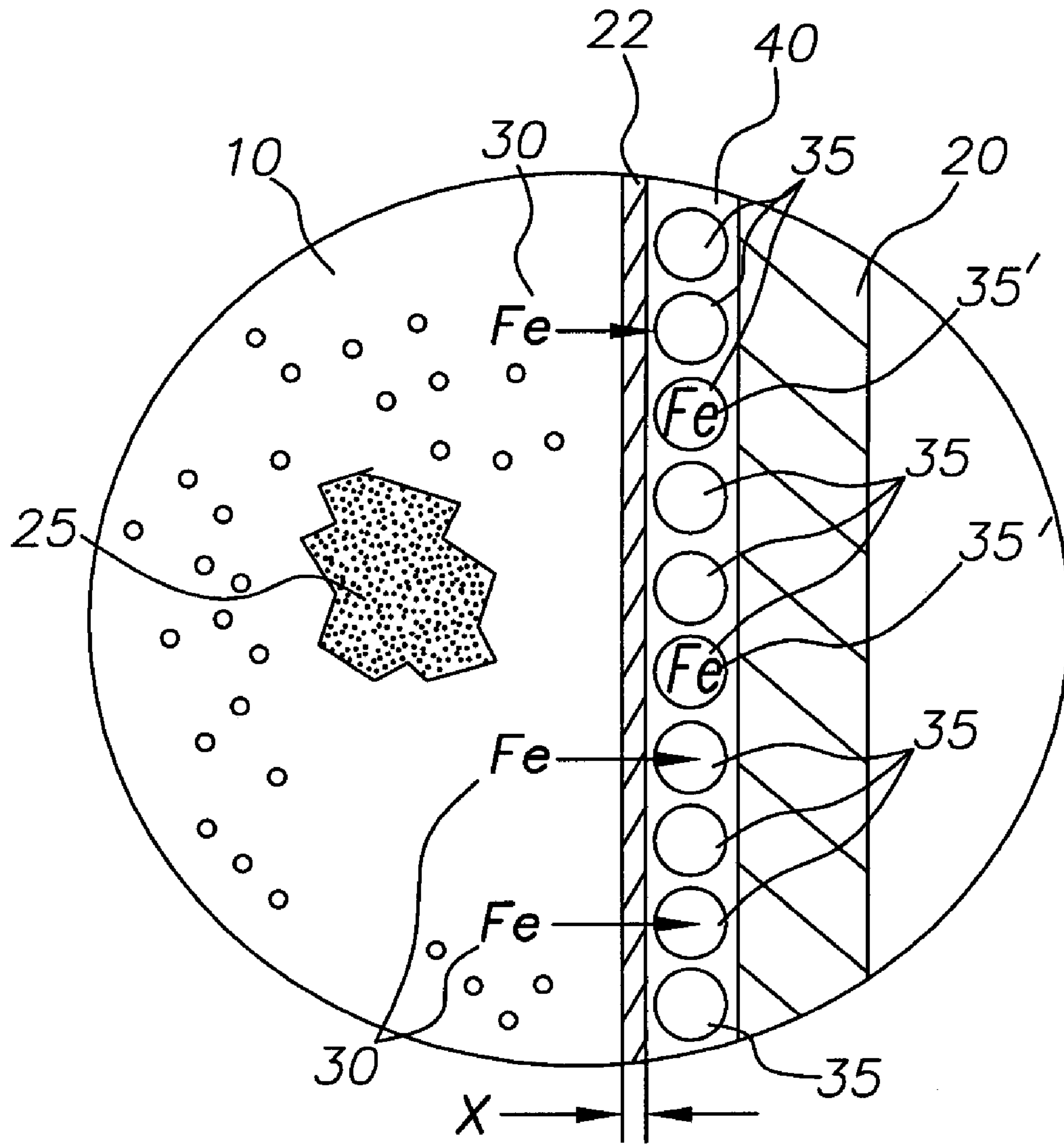


FIG. 3

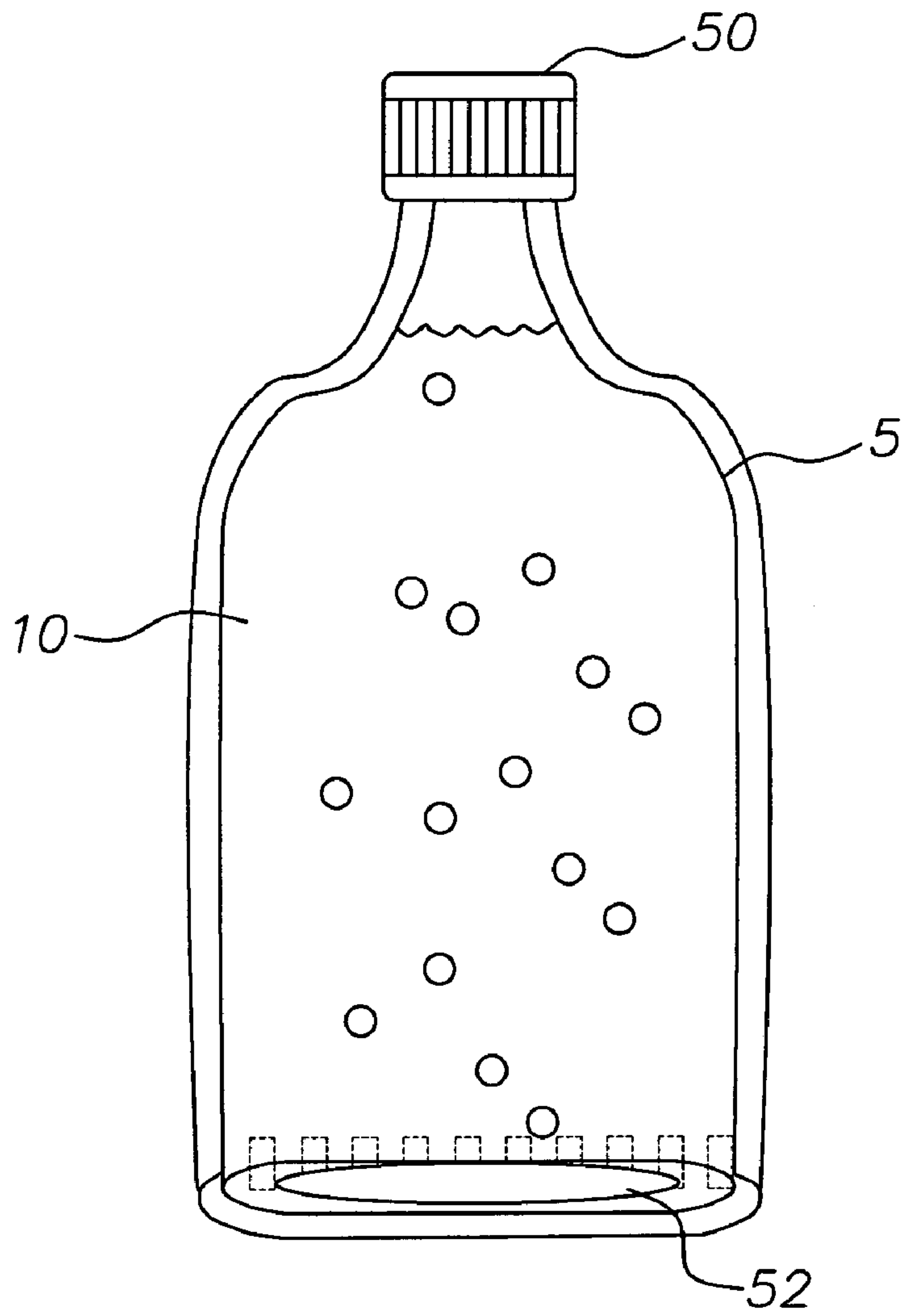


FIG. 4

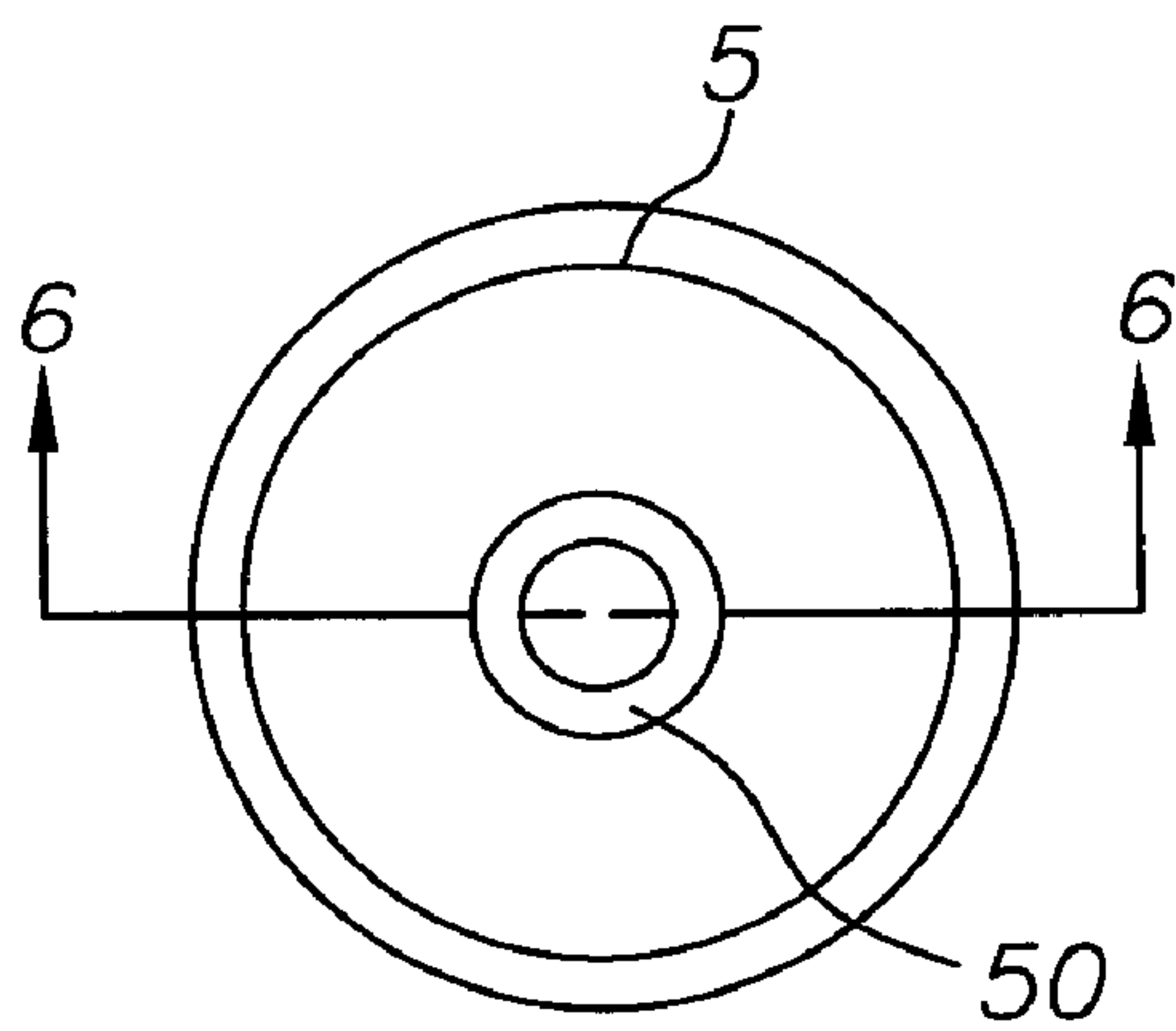


FIG. 5



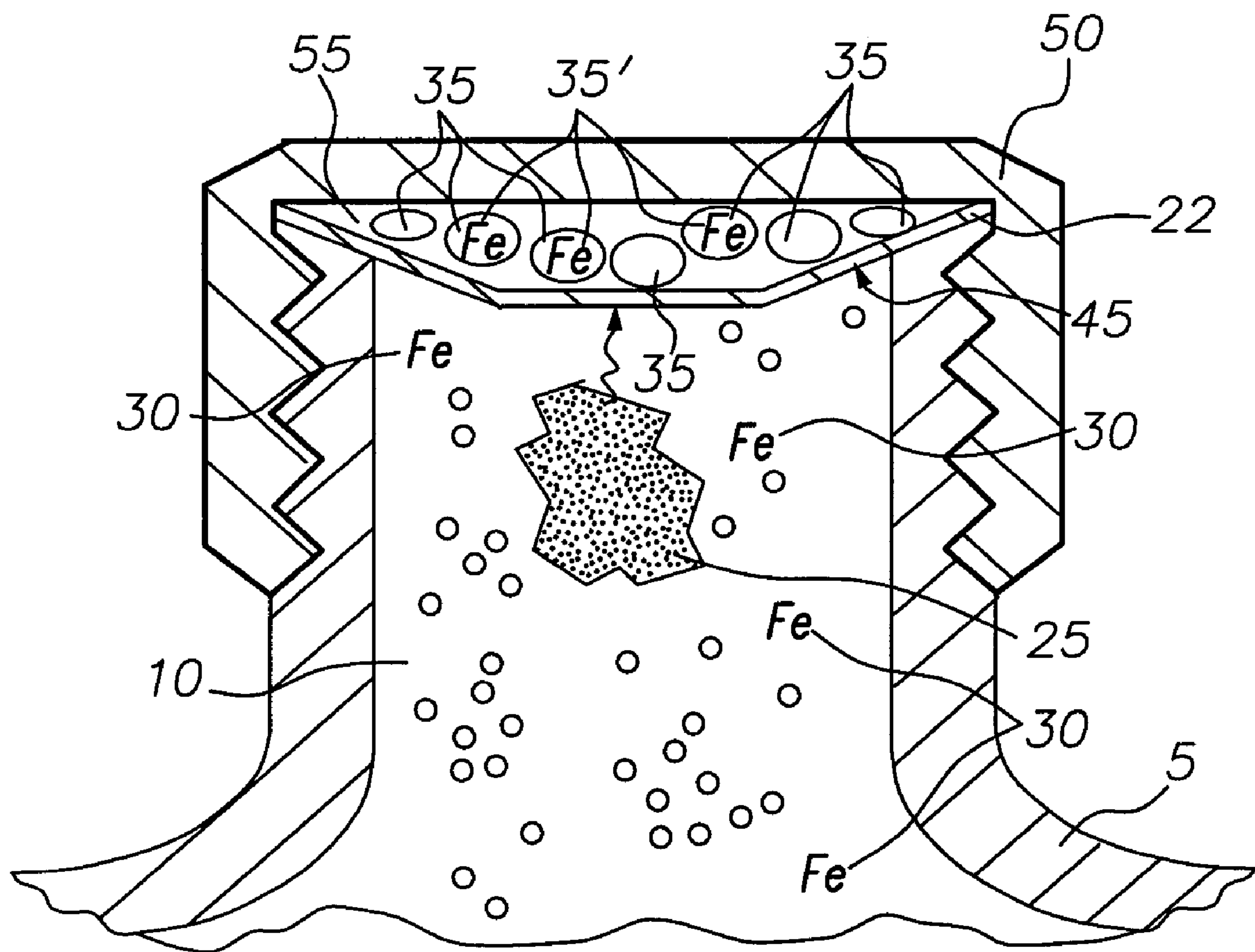


FIG. 6

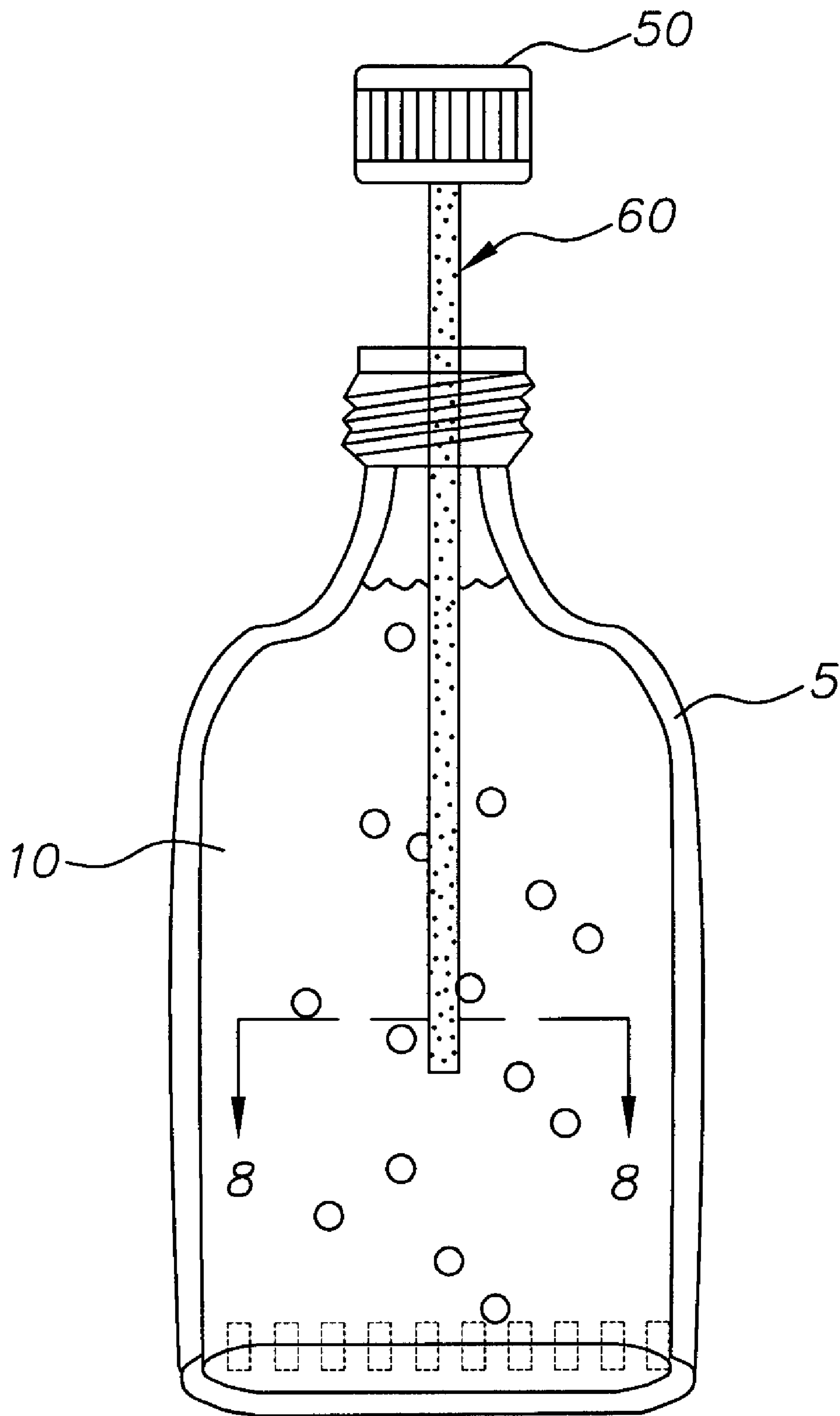


FIG. 7

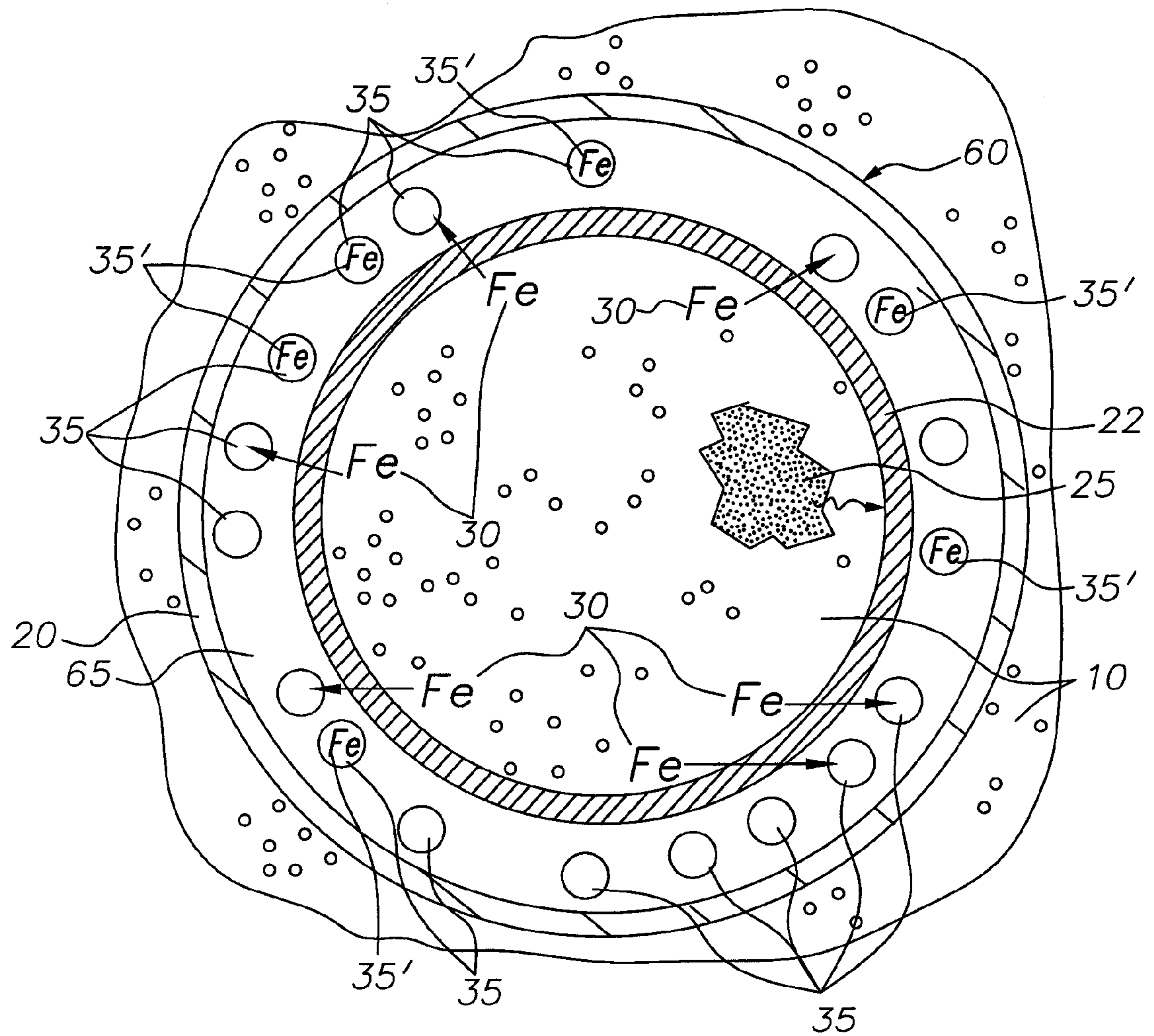


FIG. 8



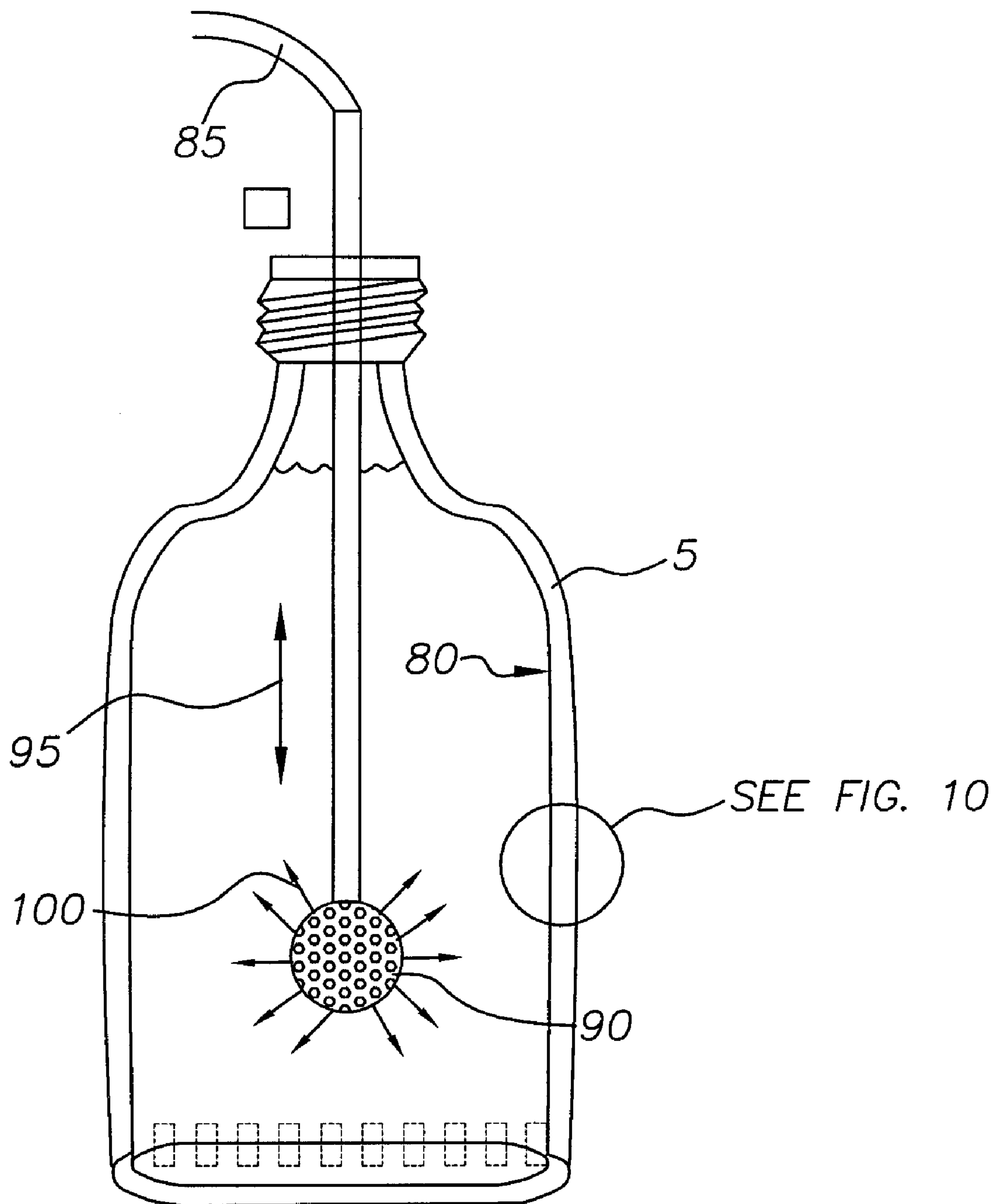


FIG. 9

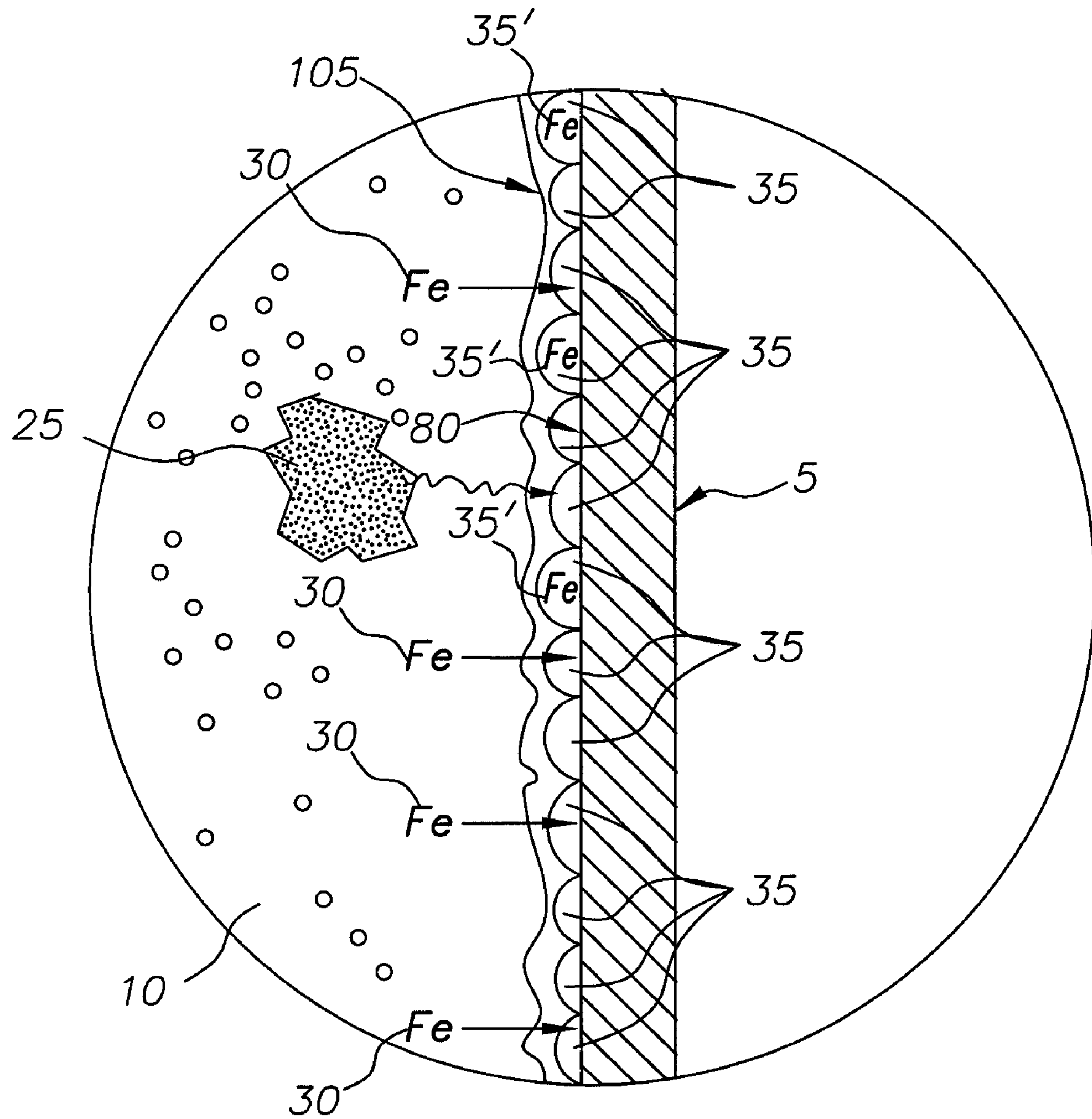


FIG. 10

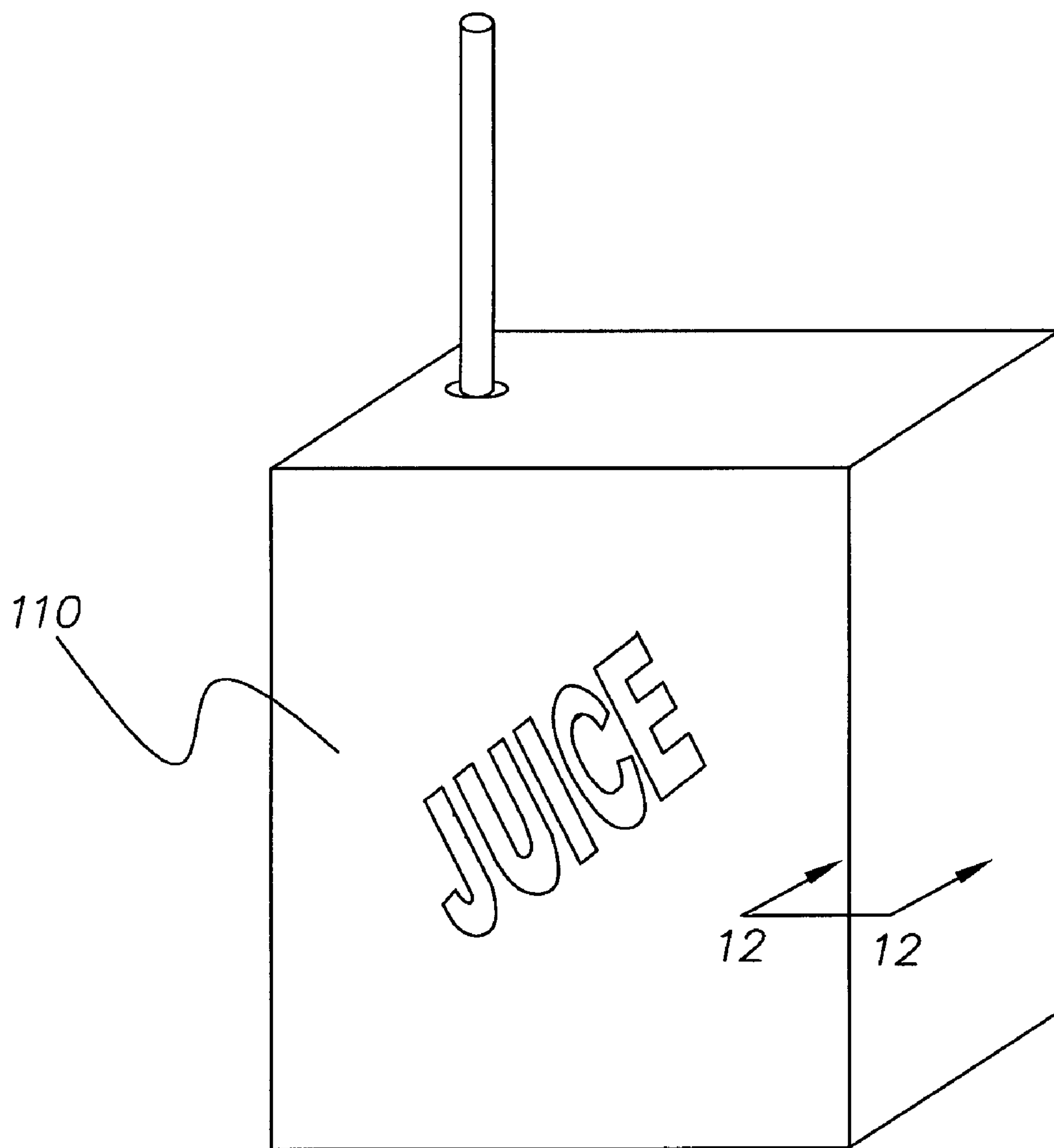


FIG. 11

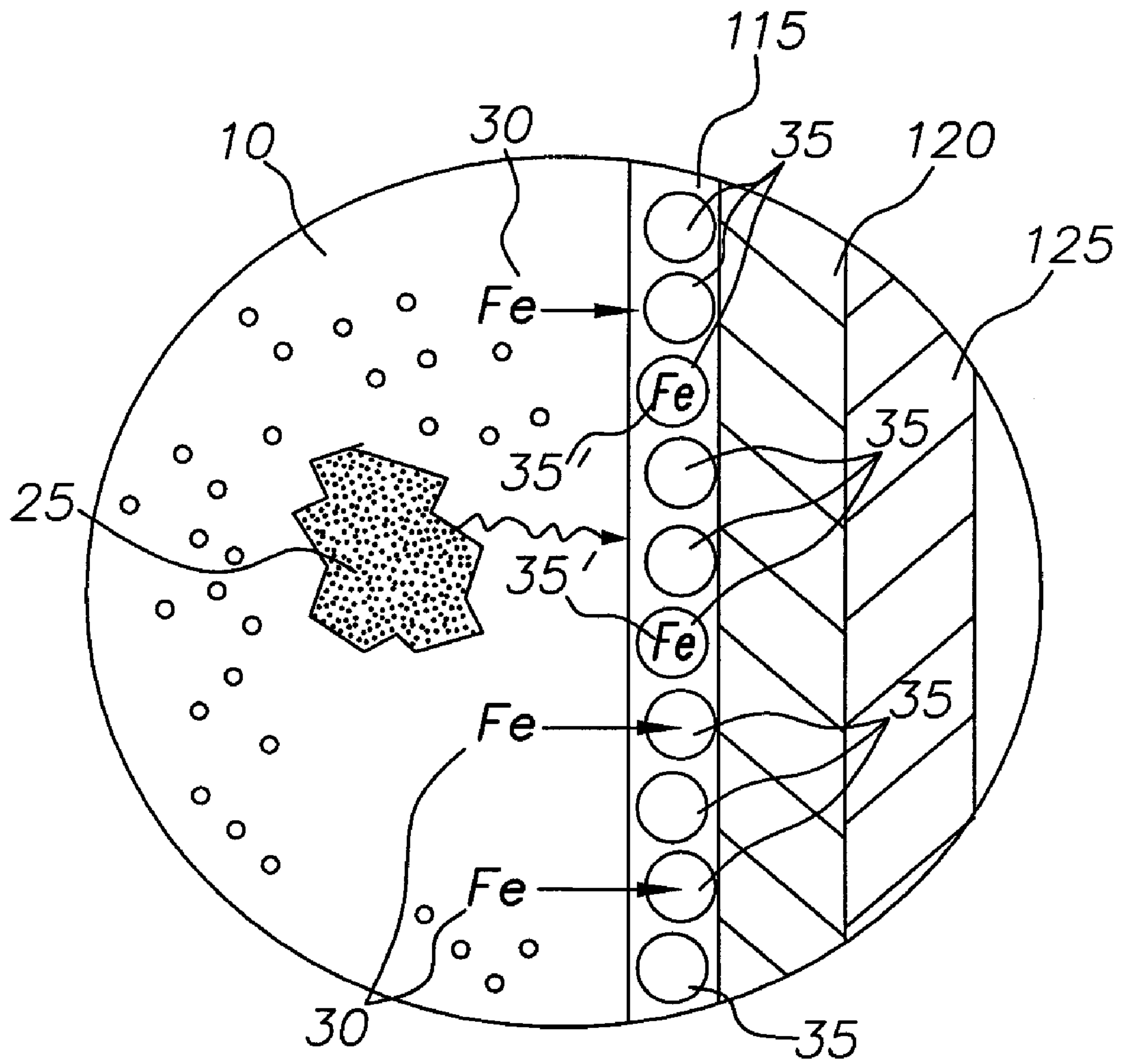


FIG. 12

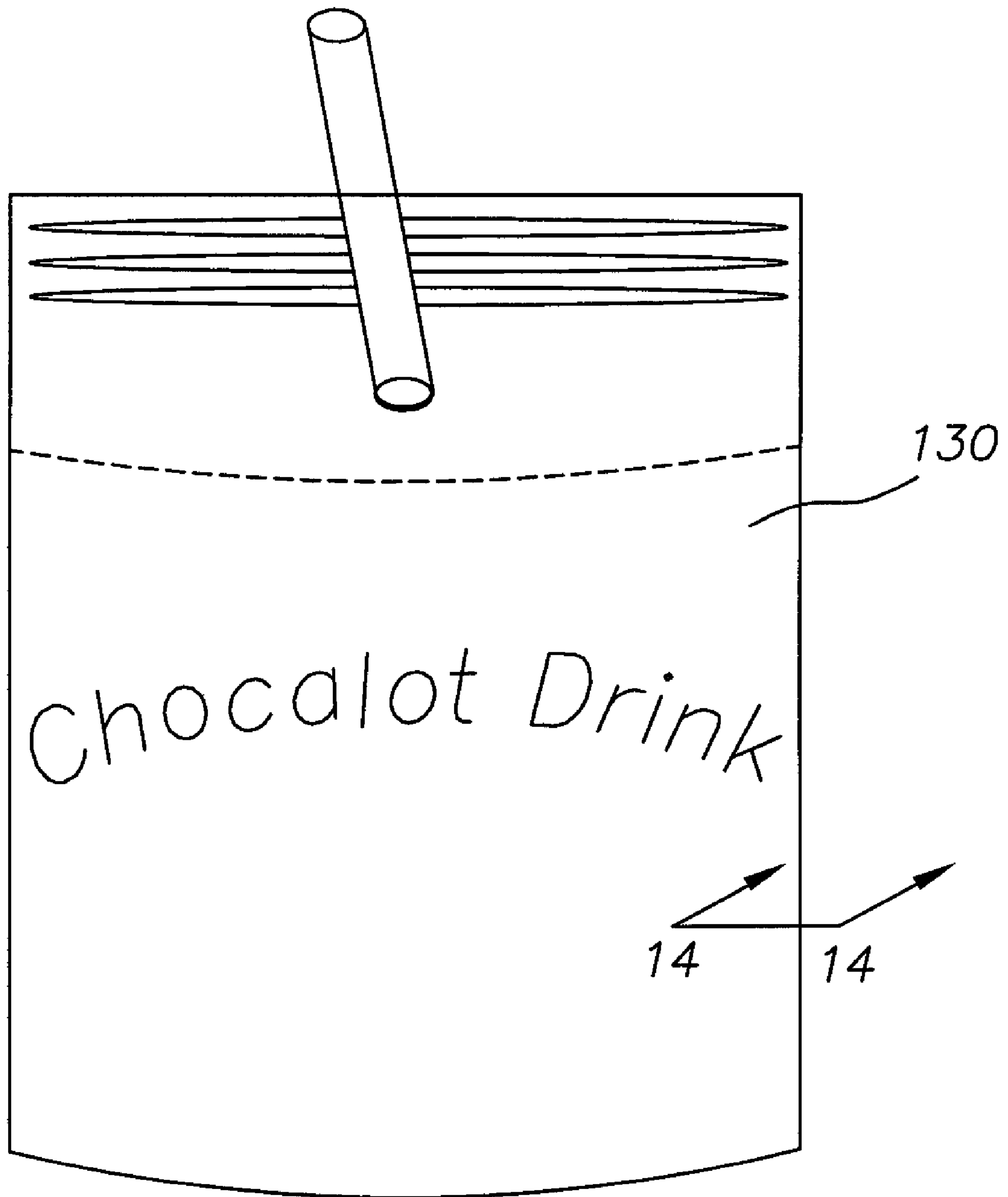


FIG. 13



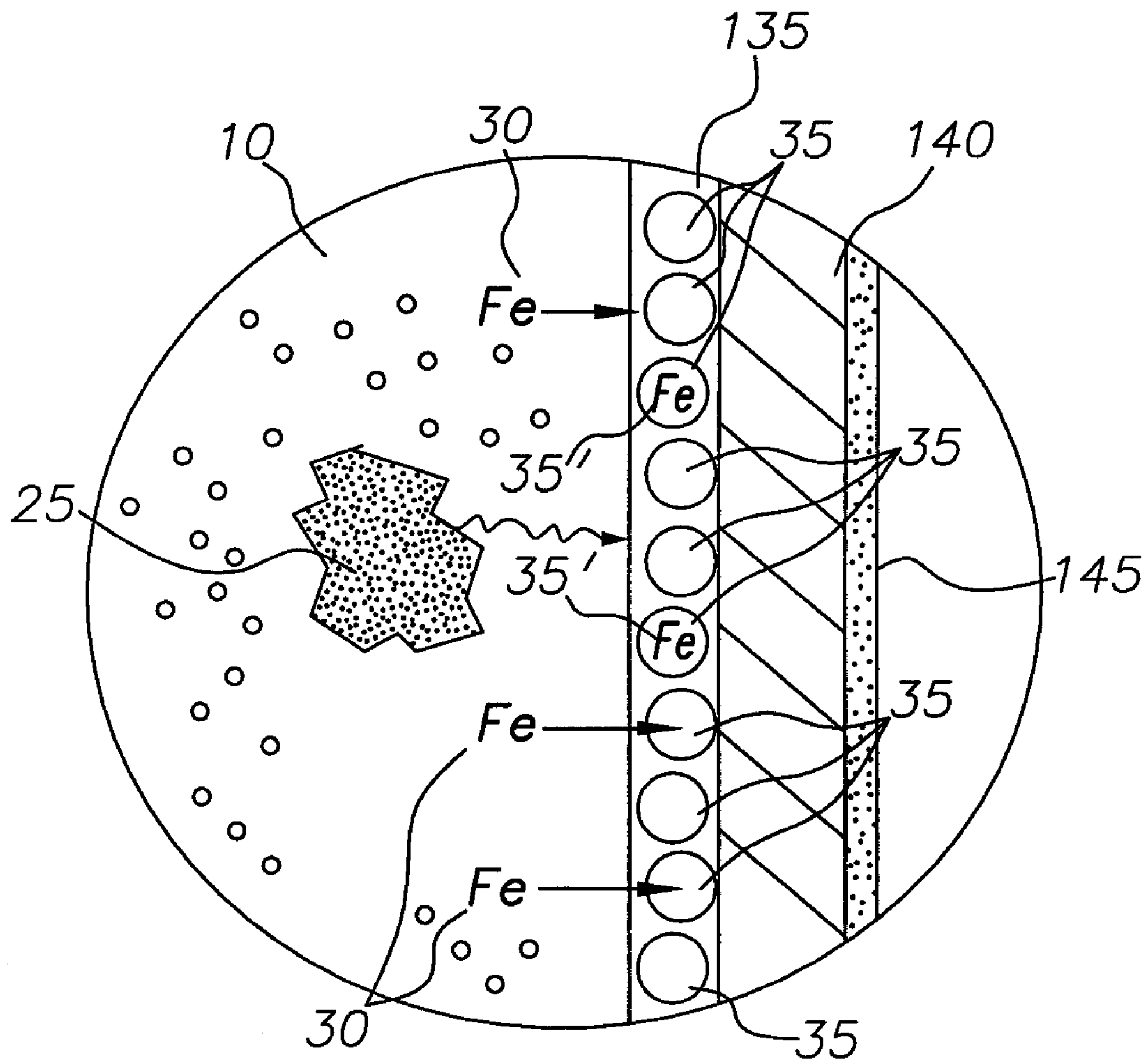


FIG. 14

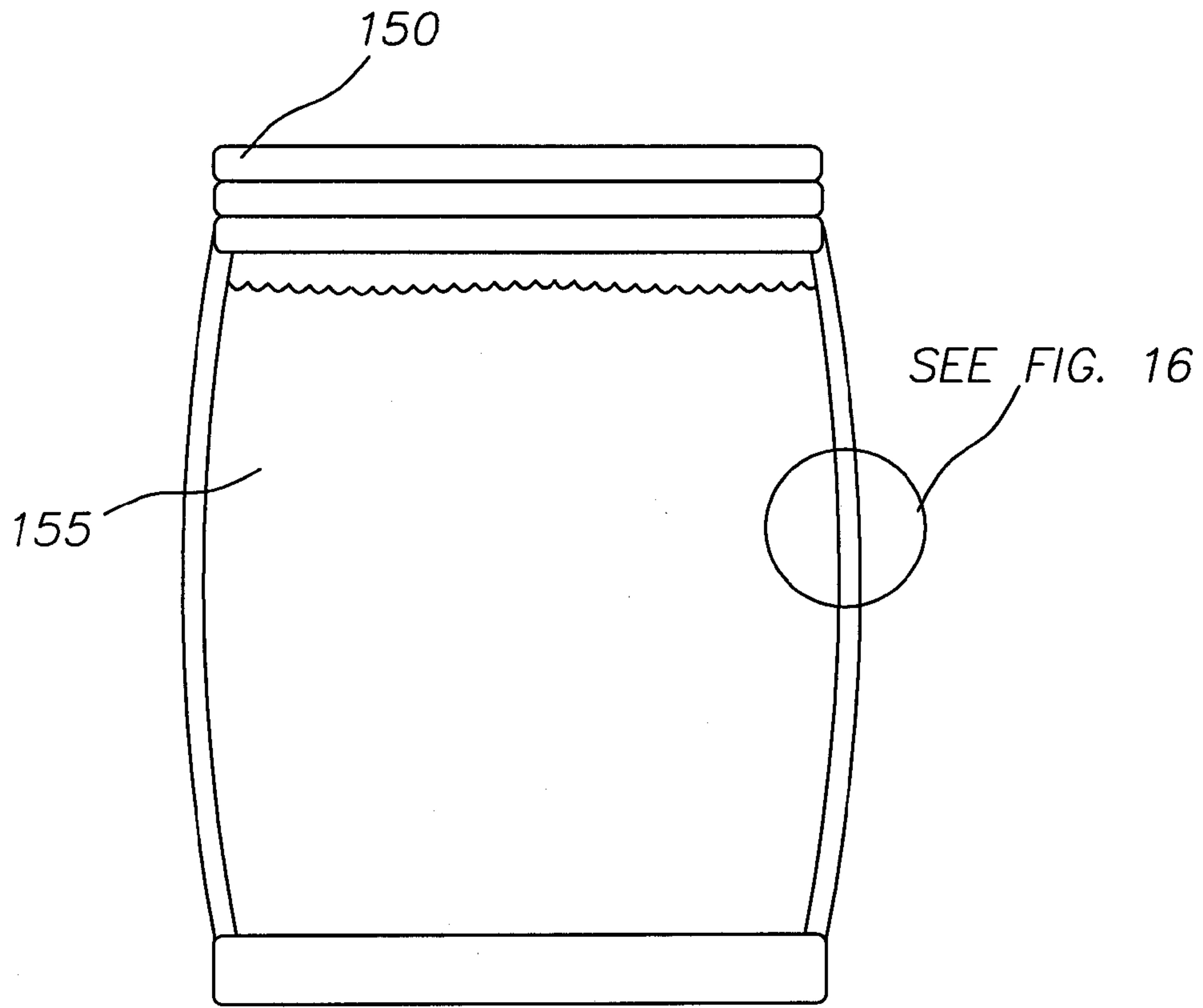


FIG. 15

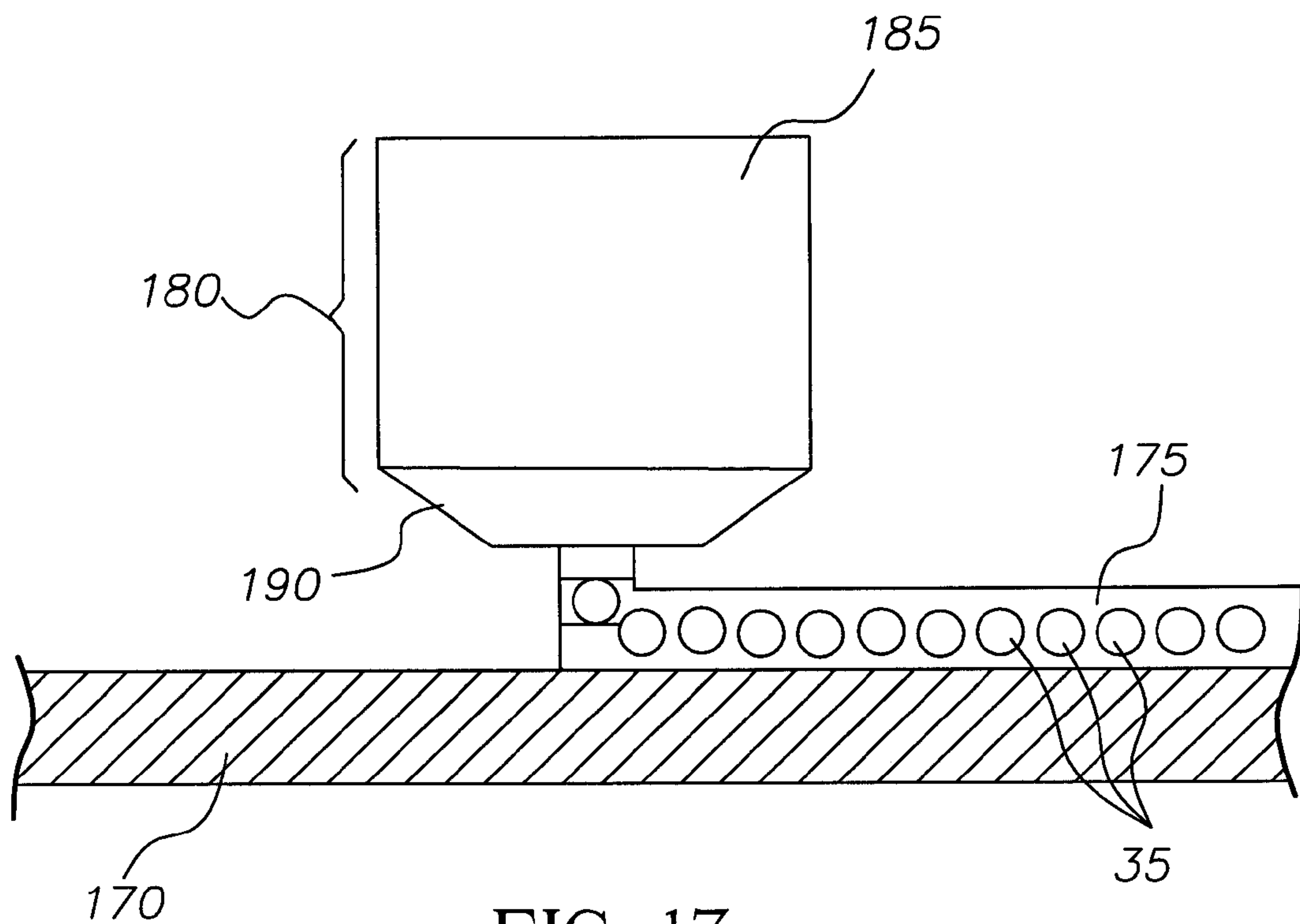


FIG. 17

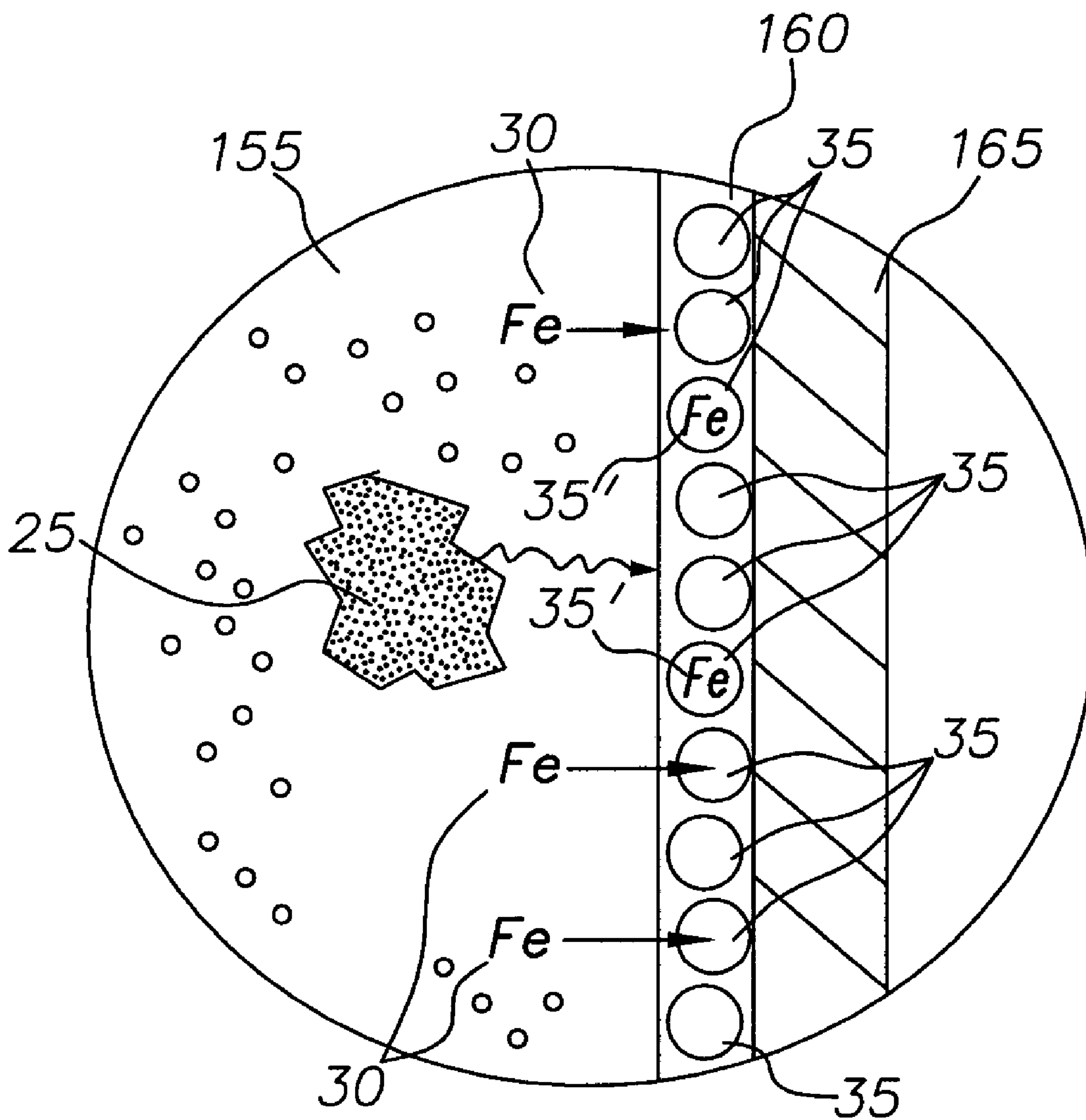


FIG. 16

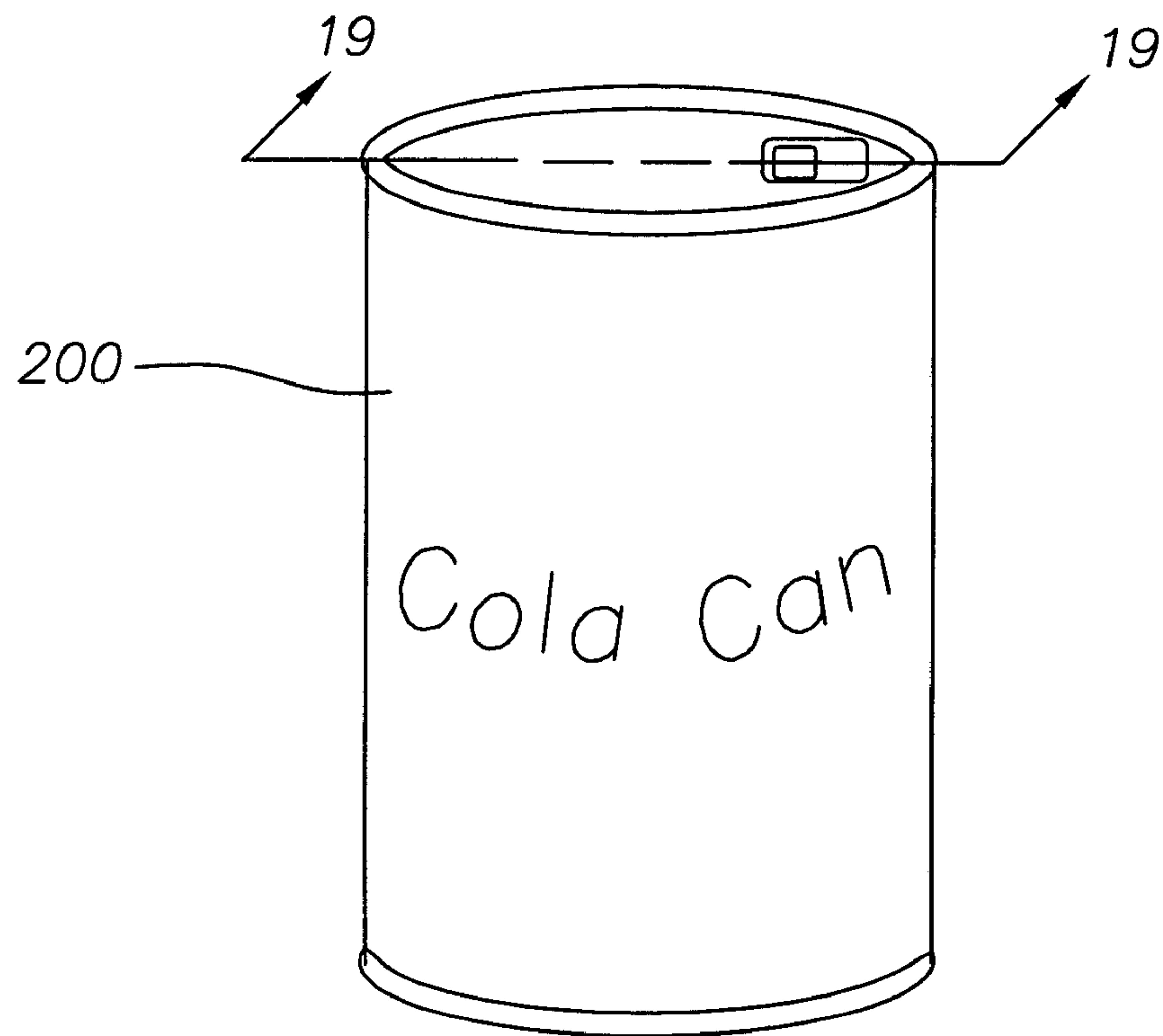


FIG. 18

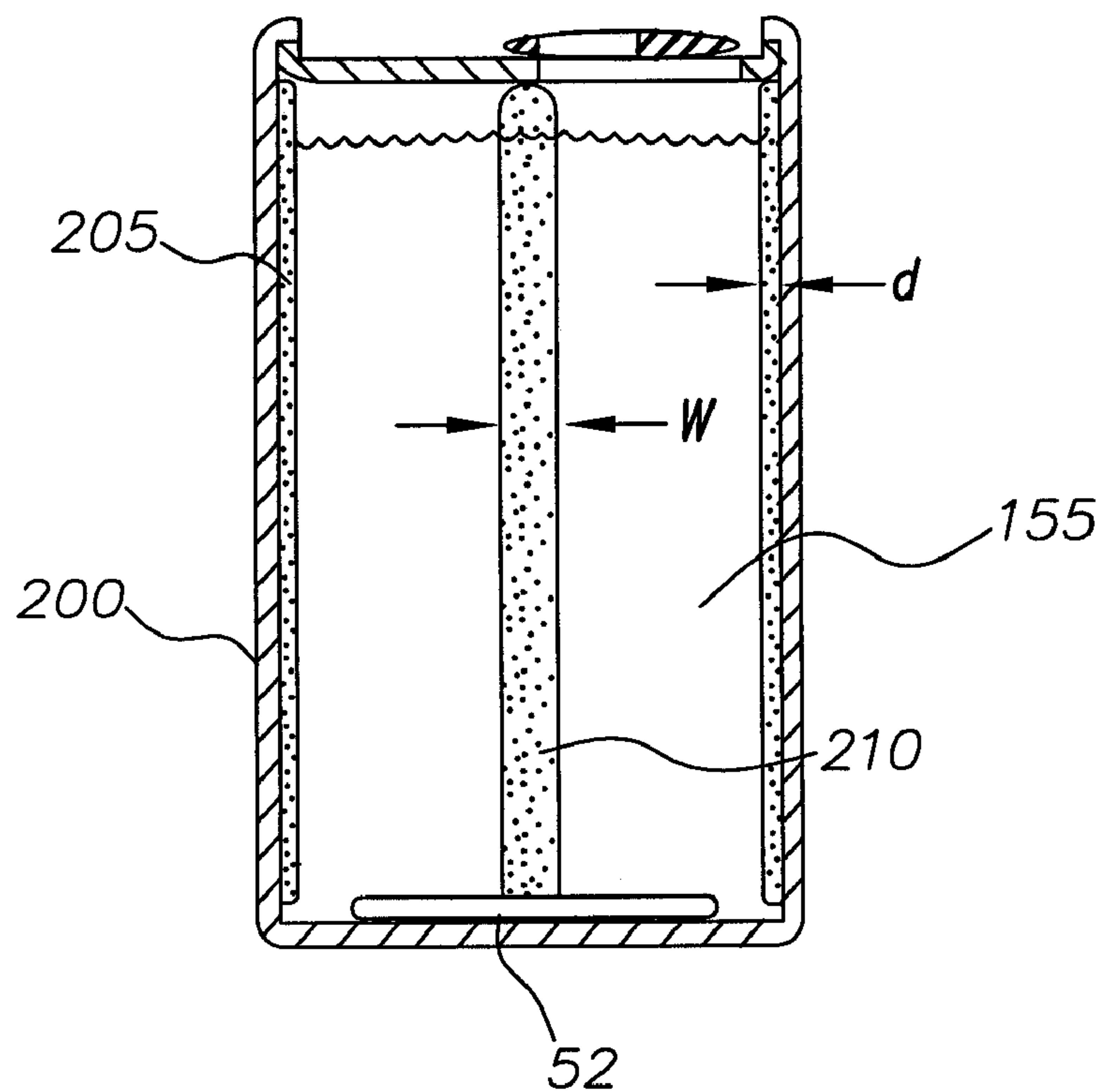


FIG. 19

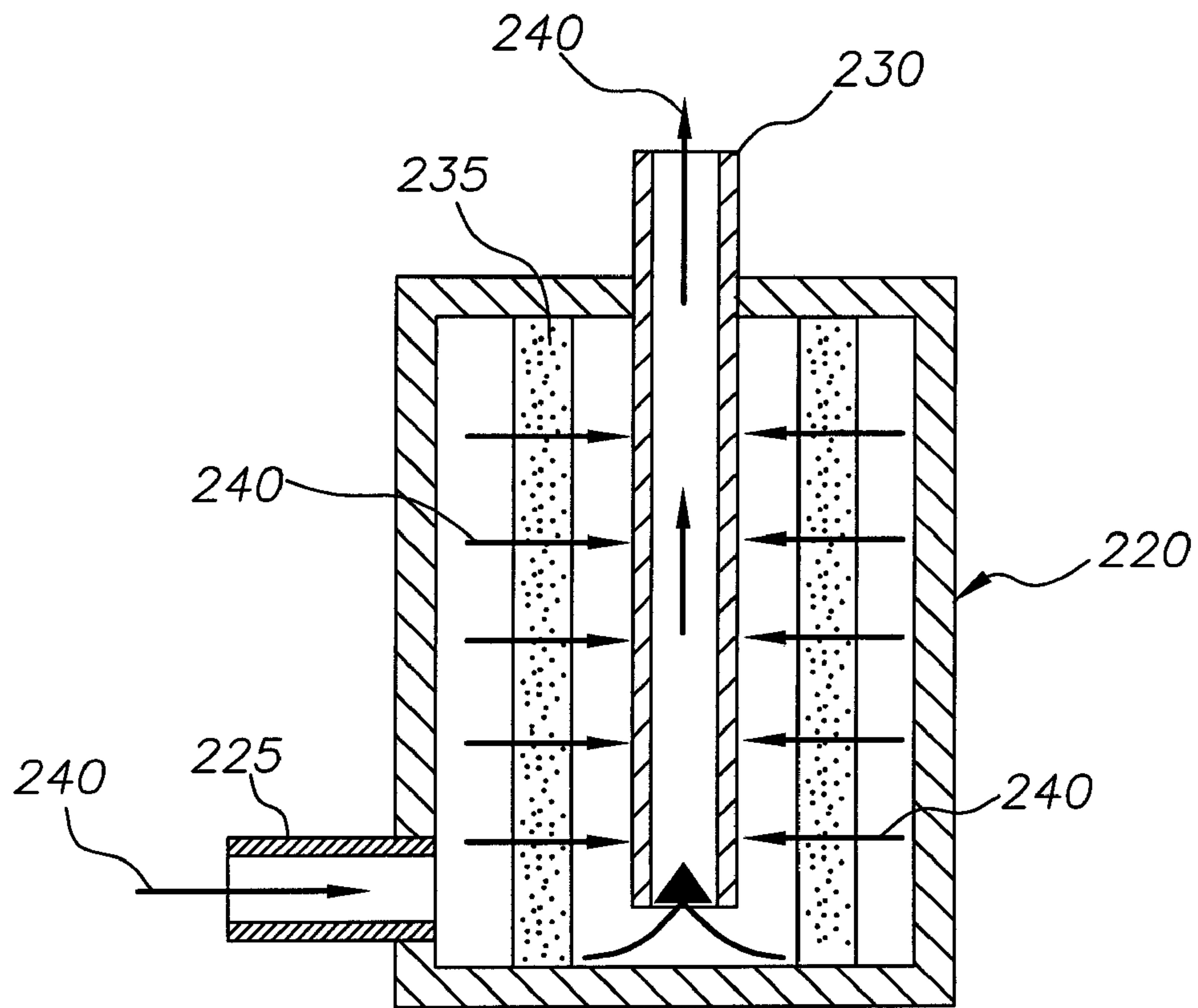


FIG. 20

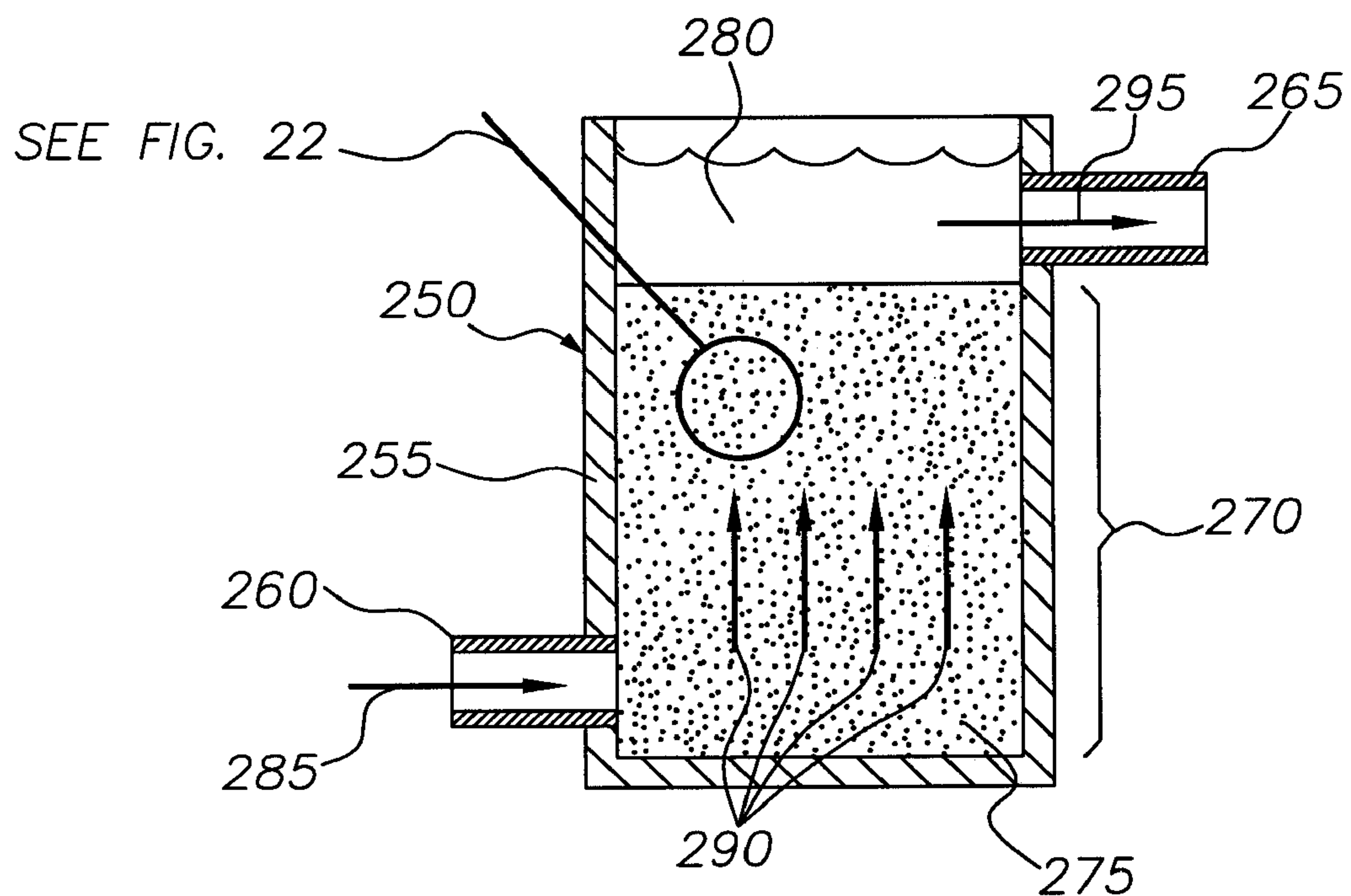


FIG. 21



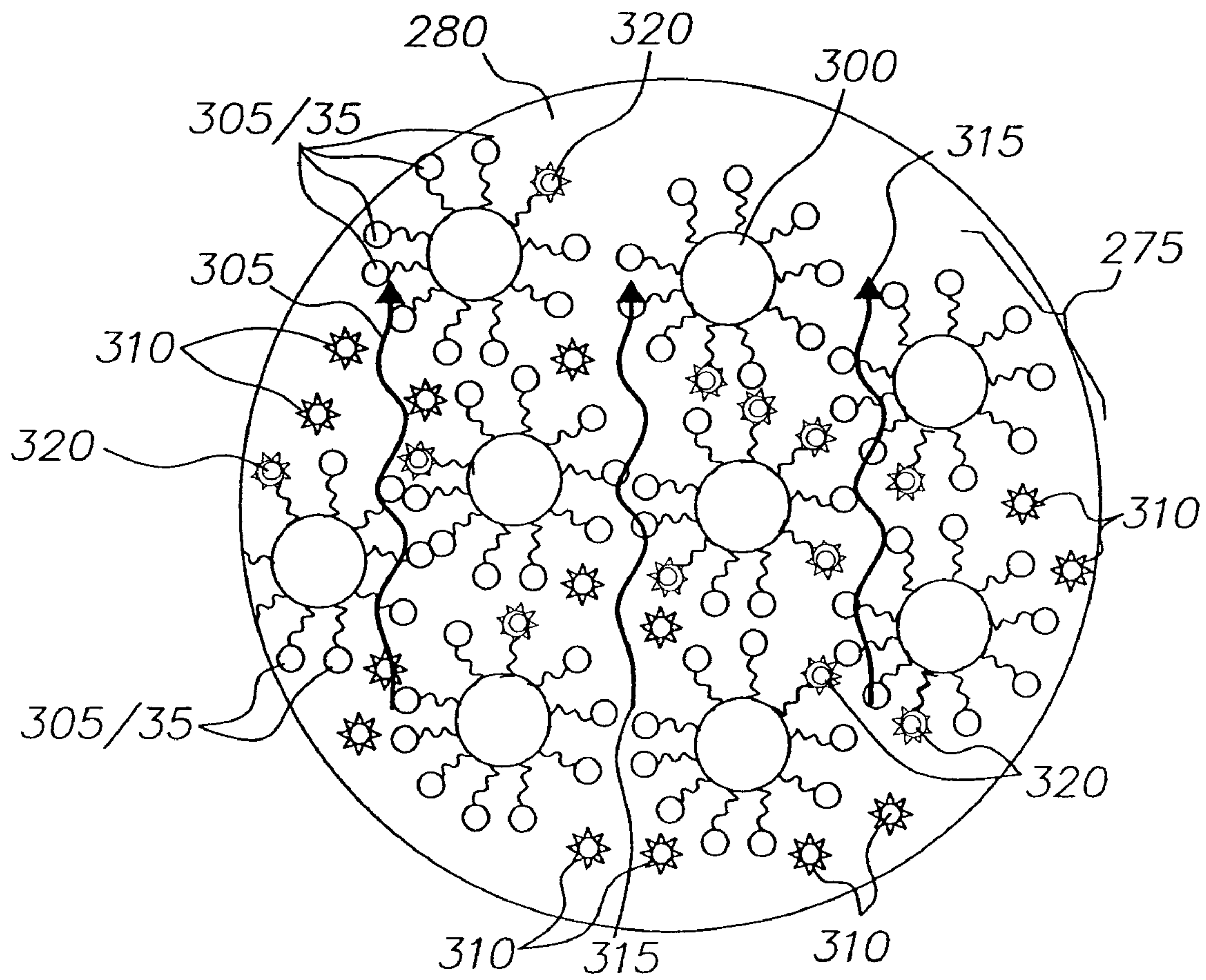


FIG. 22

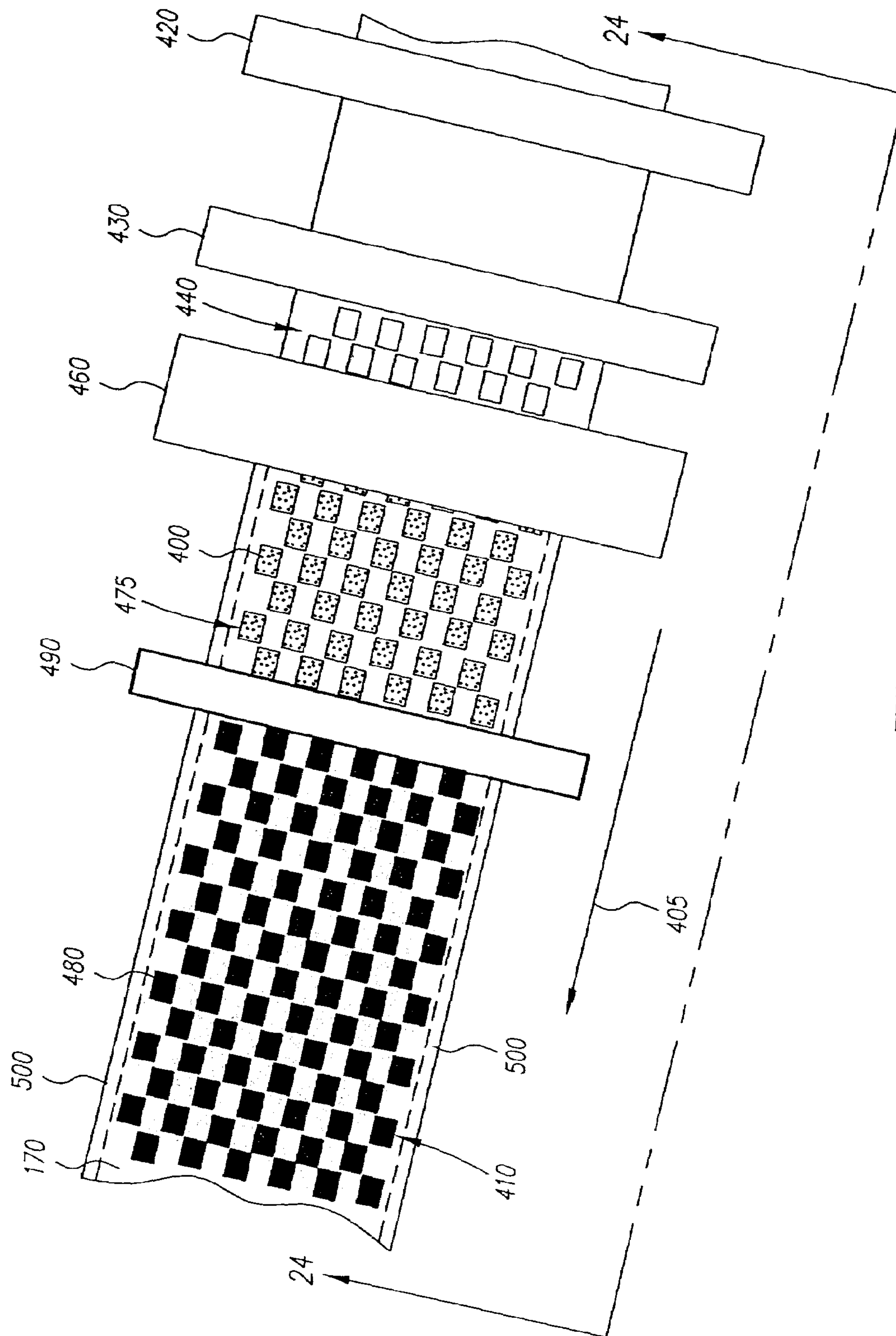


FIG. 23



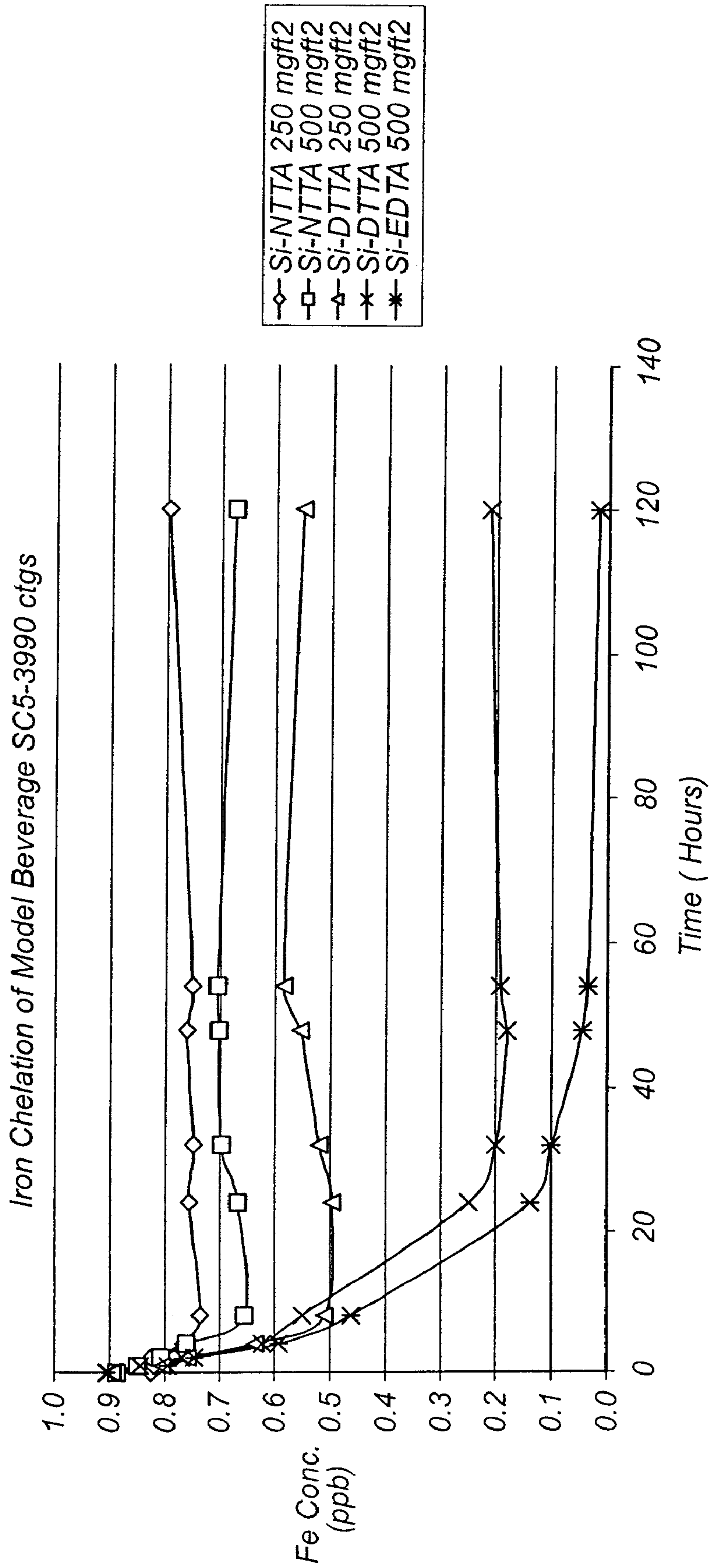


FIG. 25

Rates of Iron Removal from 17.2% w/w Ocean Spray Apple Juice Concentrate (100g) using 200 Molar Equivalents of Chelator-Functionalized Crosfield IJ624 Silica Gel

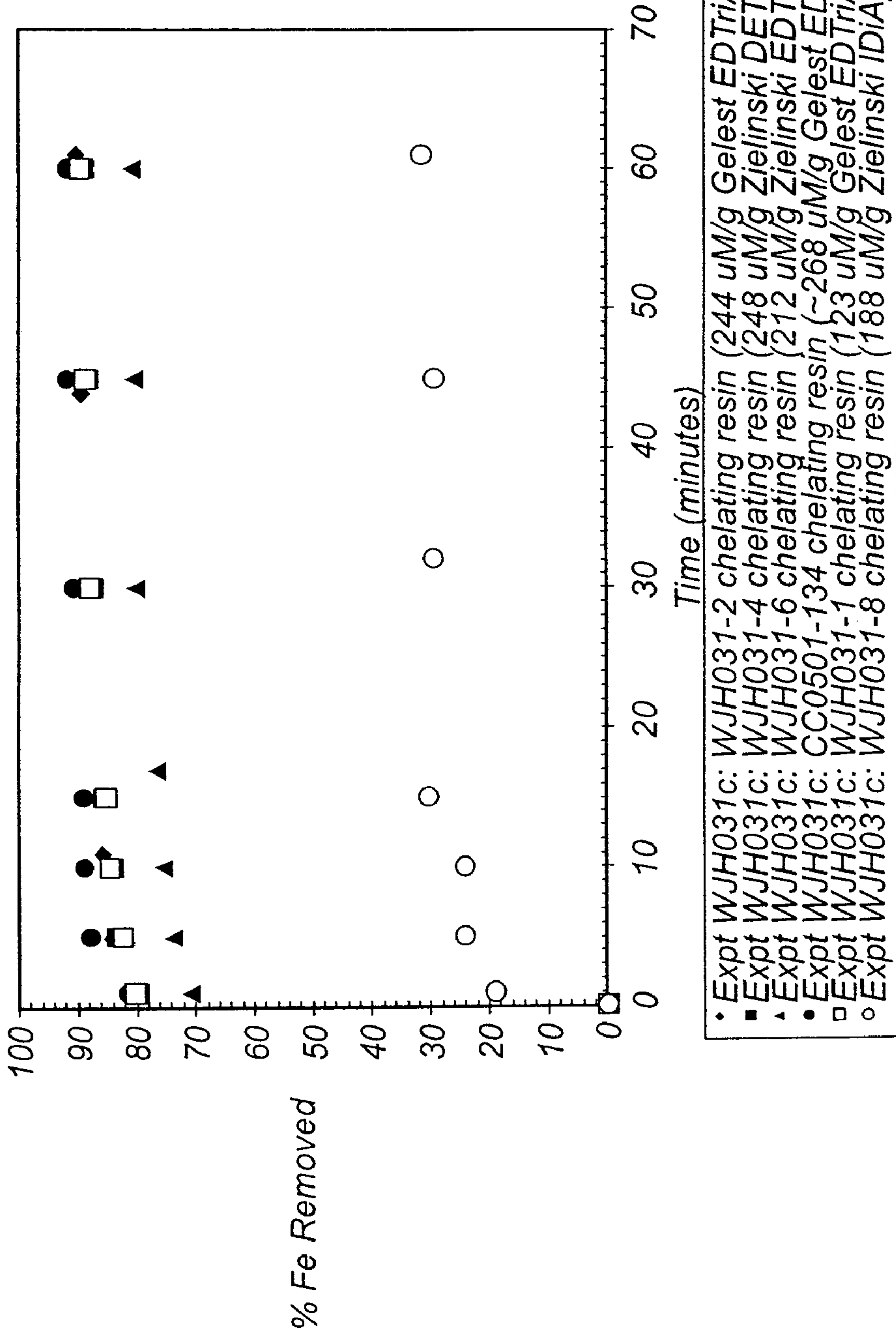


FIG. 26



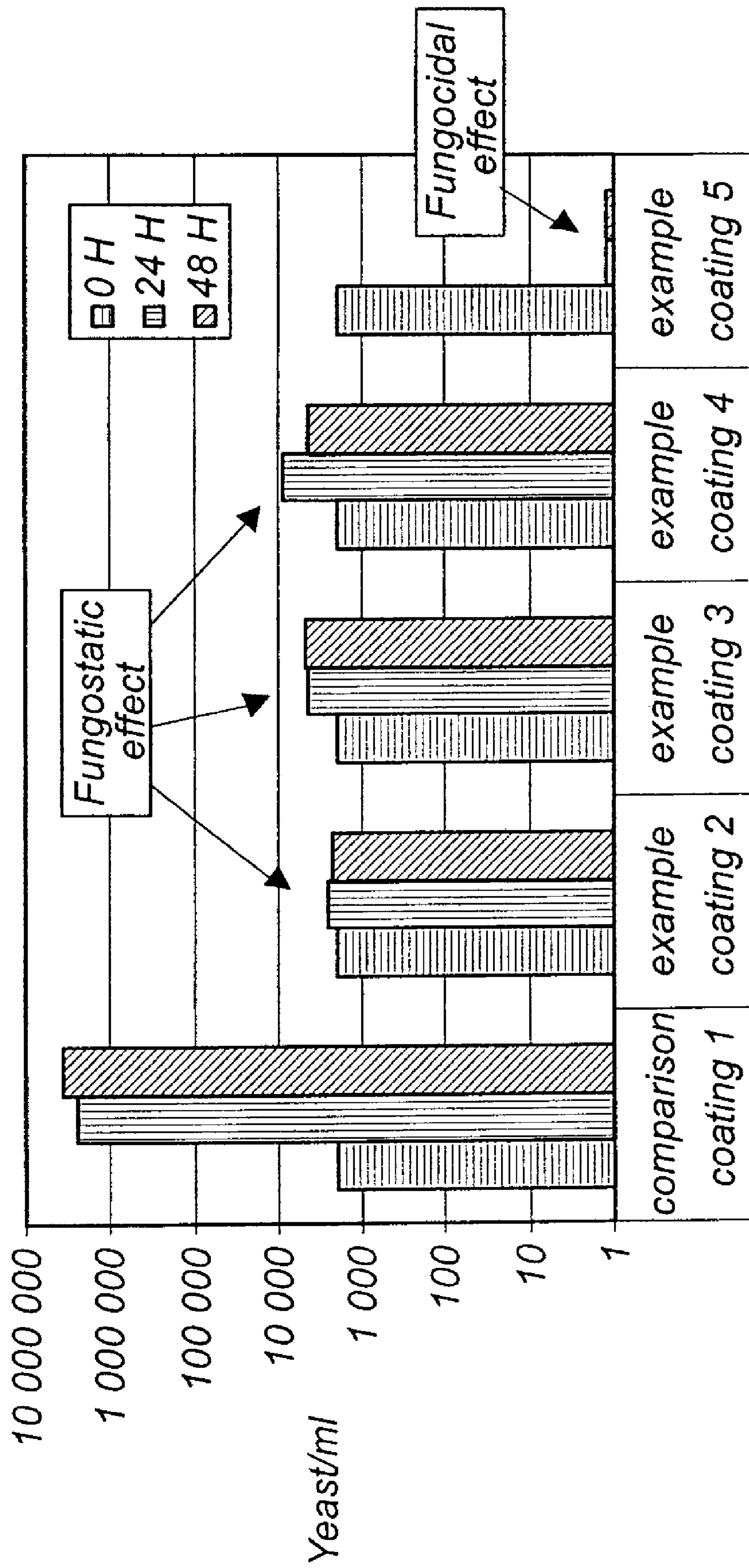


FIG. 27

## METHOD FOR INHIBITING MICROBIAL GROWTH IN LIQUID NUTRIENTS

### CROSS REFERENCE TO RELATED APPLICATIONS

This is a Continuation-in-Part of U.S. patent application Ser. No. 10/936,929 filed Sep. 9, 2004 entitled CONTAINER FOR INHIBITING MICROBIAL GROWTH IN LIQUID NUTRIENTS by David L. Patton et al., which is a Continuation-in-Part of application Ser. No. 10/823,446 filed Apr. 13, 2004 now U.S. Pat. No. 7,258,786 entitled CONTAINER FOR INHIBITING MICROBIAL GROWTH IN LIQUID NUTRIENTS by David L. Patton, et al.

Reference is also made to commonly assigned pending U.S. patent application Ser. No. 10/985,378 filed Nov. 10, 2004 entitled CONTAINER FOR INHIBITING MICROBIAL GROWTH IN LIQUID NUTRIENTS by David L. Patton, Joseph F. Bringley, Richard W. Wien, John M. Pochan, Yannick J. F. Lerat and Narashimharao Dontula and pending U.S. patent application Ser. No. 10/985,377 filed Nov. 10, 2004 entitled CONTAINER FOR INHIBITING MICROBIAL GROWTH IN LIQUID NUTRIENTS by David L. Patton, Joseph F. Bringley, Richard W. Wien, John M. Pochan, Yannick J. F. Lerat and William J. Harrison the disclosures of which are incorporated herein by reference.

### FIELD OF THE INVENTION

The present invention relates to a fluid container having a metal-ion sequestering agent for removing bio-essential metal ions from a liquid nutrient for inhibiting growth of microbes in the liquid nutrient.

### BACKGROUND OF THE INVENTION

It has been recognized that small concentrations of metal ions play an important role in biological processes. For example, Mn, Mg, Fe, Ca, Zn, Cu and Al are essential bio-metals, and are required for most, if not all, living systems. Metal ions play a crucial role in oxygen transport in living systems, and regulate the function of genes and replication in many cellular systems. Calcium is an important structural element in the life of bacteria regulating enzyme activity. Mn, Mg, Cu and Fe are involved in metabolism and enzymatic processes. At high concentrations, metals may become toxic to living systems and the organism may experience disease or illness if the level cannot be controlled. As a result the availability and concentrations, of metal ions in biological environments is a major factor in determining the abundance, growth-rate and health of plant, animal and micro-organism populations.

It has also been recognized that iron is an essential biological element, and that all living organisms require iron for survival and replication. Although, the occurrence and concentration of iron is relatively high on the earth's surface, the availability of "free" iron is severely limited by the extreme insolubility of iron in aqueous environments. As a result, many organisms have developed complex methods of procuring "free" iron for survival and replication.

Articles, such as food and beverage containers are needed that are able to improve food quality, to increase shelf-life, to protect from microbial contamination, and to do so in a manner that is safe for the user of such items and that is environmentally clean while providing for the general safety and health of the public. Materials and methods are needed to prepare articles having antimicrobial properties that are

less, or not, susceptible to microbial resistance. Methods are needed that are able to target and remove specific, biologically important, metal ions while leaving intact the concentrations of beneficial metal ions.

During the process of filling containers with certain beverages and foods, air borne pathogens enter the containers after the flash pasteurization or pasteurization part of the process. These pathogens such as yeast, spores, bacteria, etc. will grow in the nutrient rich beverage or food, ruining the taste or even causing hazardous microbiological contamination. While some beverages are packaged by aseptic means or by utilizing preservatives, many other beverages, for example fruit juices, teas and isotonic drinks are "hot-filled". "Hot-filling" involves the filling of a container with a liquid beverage having some elevated temperature (typically at about 180-200° F.). The container is capped and allowed to cool, producing a partial vacuum therein. The process of hot filling of beverages and foods is used to kill the pathogens which enter the container during the filling of the beverage or food containers. Hot filling requires containers be made of certain materials or constructed in a certain fashion such as thicker walls to withstand the hot filling process. The energy required for hot filling adds to the cost of the filling process. Temperatures required for hot filling have a detrimental effect on the flavor of the beverage. Other methods of filling such as aseptic filling require large capital expenditures and maintaining class 5 clean room conditions.

U.S. Pat. No. 5,854,303 discloses a polymeric material incorporating a polyvalent cation chelating agent in an amount effective to inhibit the growth of a protozoan on the surface of contact lenses and in other eye care products.

### PROBLEM TO BE SOLVED BY THE INVENTION

The present invention is directed to the problem of the growth of micro-organism in liquids that adversely affects food quality, shelf-life, and to protect the liquid from microbial contamination by providing storage/holding tanks or passed the liquid through a filter device.

### SUMMARY OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a method for inhibiting growth of microbes in a liquid having a pH equal to or greater than about 2.5, comprising the steps of:

a. providing a filter bed assembly for filtering the liquid, the filter bed assembly having a metal-ion sequestering agent for removing designated metal ion from the liquid, the filter assembly having an inlet and an outlet for allowing the liquid to enter and leave the filter assembly; and

b. causing the liquid to enter the fluid bed assembly through an inlet pass through the filter bed and out an outlet for a sufficient time so as to substantially reduce the designated metal ion from the liquid.

In accordance with another aspect of the present invention there is provided a method for inhibiting growth of microbes in a liquid having a pH equal to or greater than about 2.5, comprising the steps of:

a. providing a filter assembly for filtering the liquid, the filter assembly having a filter having a metal-ion sequestering agent for removing designated metal ion from the liquid, the filter assembly having an inlet and an outlet for allowing the liquid to enter and leave the filter assembly; and



b. causing the liquid to enter the filter assembly through the inlet pass through the filter and out the outlet for a sufficient time so as to substantially reduce the designated metal ion from the liquid.

These and other aspects, objects, features and advantages of the present invention will be more clearly understood and appreciated from a review of the following detailed description of the preferred embodiments and appended claims and by reference to the accompanying drawings.

#### BRIEF DESCRIPTION OF THE DRAWINGS

In the detailed description of the preferred embodiments of the invention presented below, reference is made to the accompanying drawings in which:

FIG. 1 illustrates a cross section of a fluid container made in accordance with the prior art;

FIG. 2 is an enlarged partial cross sectional view of a portion of the container of FIG. 1 illustrating a "free" iron ion sequestering agent;

FIG. 3 is a view similar to FIG. 2 illustrating a container made in accordance with the present invention;

FIG. 4 illustrates a bottle with a bottle cap also made in accordance with the present invention;

FIG. 5 is a schematic top plan view of the bottle and cap of FIG. 4;

FIG. 6 is an enlarged partial cross sectional view of the bottle and cap taken along line 6-6 of FIG. 5;

FIG. 7 is a schematic view of a projecting member extending from a modified cap of FIG. 5 also made in accordance with the present invention;

FIG. 8 is an enlarged cross sectional view of the projecting member of FIG. 7 as taken along line 8-8;

FIG. 9 is a schematic view of another embodiment of the present invention illustrating one method for applying a coating to the interior surface of a bottle made in accordance with the present invention;

FIG. 10 is an enlarged partial cross sectional view of a portion of the bottle of FIG. 9 illustrating the sprayed coating of the ion sequestering agent;

FIG. 11 is a schematic view of another fluid container made in accordance with the present invention such as a juice box;

FIG. 12 is an enlarged partial cross sectional view of the juice box taken along line 12-12 of FIG. 11;

FIG. 13 is a schematic view of yet another fluid container such as a stand up pouch made in accordance with the present invention;

FIG. 14 is an enlarged partial cross sectional view of the stand up pouch taken along line 14-14 of FIG. 13;

FIG. 15 is a schematic view of still another embodiment of a fluid container such as a bag also made in accordance with the present invention;

FIG. 16 is an enlarged partial cross sectional view of a portion of the bag of FIG. 15 as indicated by circle 16;

FIG. 17 is a cross-sectional view of a web that can be used in the manufacture of a box, pouch or bag showing a coating assembly for coating a hydrophilic layer containing a metal-ion sequestering agent;

FIG. 18 is a schematic view of yet another fluid container, such as a can, made in accordance with the present invention;

FIG. 19 is a cross sectional view of FIG. 18 as taken along line 19-19;

FIG. 20 is a cross sectional view of a filter assembly made in accordance with the present invention;

FIG. 21 is a cross sectional view of a fluid bed ion exchange assembly made in accordance with the present invention; and

FIG. 22 is an enlarged partial view of a portion of the fluid bed ion exchange assembly of FIG. 21 as identified by circle 22 illustrating a metal-ion sequestering agent.

FIG. 23 is a schematic top view of the web and device of FIG. 24.

FIG. 24 is a schematic view of electro-photographic device for applying a pattern of metal ion chelating agents to a web, such as is used to make a box, pouch or bag in accordance with the present invention.

FIG. 25 is a graph illustrating iron chelation of model beverages.

FIG. 26 is a graph illustrating rates of iron removal from 17.2% w/w Ocean Spray™ Apple Juice Concentrate.

FIG. 27 is a bar graph illustrating fungostatic and fungicidal effects.

#### DETAILED DESCRIPTION OF THE INVENTION

The growth of microbes in an article such as a fluid container containing a liquid nutrient comprising a liquid nutrient can be inhibited by placing metal-ion sequestering agents, as described in U.S. patent application Ser. No. 10/822,940 filed Apr. 13, 2004 entitled DERIVATIZED NANOPARTICLES COMPRISING METAL-ION SEQUESTRANT by Joseph F. Bringley, and U.S. patent application Ser. No. 10/822,929 filed Apr. 13, 2004 entitled COMPOSITION OF MATTER COMPRISING POLYMER AND DERIVATIZED NANOPARTICLES by Joseph F. Bringley et al. capable of removing a designated metal ion for example, Mn, Mg, Fe, Ca, Zn, Cu and Al from said liquid nutrients, in contact with the nutrient. Intimate contact is achieved by incorporating the metal-ion sequestering agent as an integral part of the support structure of the article. For example, one can control the concentration of "free" iron in the liquid nutrient held by the article by placing an iron sequestering agent in the walls of the container, which in turn controls the growth rates, and abundance of micro-organisms. The articles of the invention further contain an effective amount of an antimicrobial agent, which quickly reduces the population of microbes to a manageable level, and insures the effectiveness of metal-ion sequestering or binding agents. The invention "starves" the remaining micro-organisms of minute quantities of essential nutrients (metal-ions) and hence limits their growth and reduces the risk due to bacterial, viral and other infectious diseases. The article, such as a container, may be used for holding a food or beverage.

The term inhibition of microbial-growth, or a material which "inhibits" microbial growth, is used by the authors to mean materials, which either prevent microbial growth, or subsequently kills microbes so that the population is within acceptable limits, or materials, which significantly retard the growth processes of microbes or maintain the level or microbes to a prescribed level or range. The prescribed level may vary widely depending upon the microbe and its pathogenicity; generally it is preferred that harmful organisms are present at no more than 10 organisms/ml and preferably less than 1 organism/ml. Antimicrobial agents which kill microbes or substantially reduce the population of microbes are often referred to as biocidal materials, while materials which simply slow or retard normal biological growth are referred to as biostatic materials. The preferred impact upon the microbial population may vary widely



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depending upon the application, for pathogenic organisms (such as *E. coli* O157:H7) a biocidal effect is more preferred, while for less harmful organisms a biostatic impact may be preferred. Generally, it is preferred that microbiological organisms remain at a level which is not harmful to the consumer or user of that particular article

Metal-ion sequestering agents may be incorporated into articles by placing the metal-ion sequestering agents on the surface of the article, or by putting the metal-ion sequestering agents within the materials used to form the article. In all instances, the metal-ion sequestering agents must be capable of contacting the food or beverage held by the container.

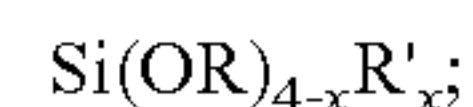
Referring to FIG. 1, there is illustrated a cross-sectional view of a typical prior art container. In the embodiment illustrated, the container comprises a bottle 5 holding a liquid nutrient 10, for example an isotonic liquid. Drinks such as Gatorade™ or PowerAde™ are examples of isotonic drinks/liquids. The container 5 may be made of one or more layers of a plastic polymer using various molding processes known by those skilled in the art. Examples of polymers used in the manufacture of bottles are PET (polyethylene terephthalate), PP (polypropylene), LDPE (low density polyethylene) and HDPE (high density polyethylene). FIG. 2 illustrates a plastic bottle 5 formed using two different polymeric layers 15 and 20. However it is to be understood that the container 5 may comprise any desired number of layers.

A fluid container made in accordance with the present invention is especially useful for containing a liquid nutrient having a pH equal to or greater than about 2.5. The container is designed to have an interior surface having a metal-ion sequestering agent for removing a designated metal ion from a liquid nutrient for inhibiting growth of microbes in said liquid nutrient. It is preferred that the metal-ion sequestering agent is immobilized within the materials forming the container or is immobilized within a polymeric layer directly in contact with the beverage or liquid nutrient. It is further preferred that the metal-ion sequestering agent is immobilized on the surface(s) of said container. This is important because metal-ion sequestrants that are not immobilized may diffuse through the material or polymeric layers of the container and dissolve into the contents of the beverage. Metal ions complexed by dissolved sequestrants will not be sequestered within the surfaces of the container but may be available for use by micro-organisms.

It is preferred that the sequestering agent is immobilized on the surface(s) of said container and has a high-affinity for biologically important metal ions such as Mn, Mg, Zn, Cu and Fe. It is further preferred that the immobilized sequestering agent has a high-selectivity for biologically important metal ions such as Mn, Mg, Zn, Cu and Fe. It is preferred that said sequestering agent has a high-selectivity for certain metal ions but a low-affinity for at least one other ion. It is further preferred that said certain metal ions comprises Mn, Mg, Zn, Cu and Fe and said other at least one ion comprises calcium. This is preferred because some metal ions such as calcium, sodium and potassium may be beneficial to the taste and quality of the food, and are usually very highly abundant in foodstuffs and in liquid extrudates of foodstuffs. It is preferred that said metal-ion sequestering agent is immobilized on the surface(s) of said container and has a stability constant greater than  $10^{10}$  with iron (III), more preferably greater than  $10^{20}$  with iron (III), and most preferably greater than  $10^{30}$  with iron (III). This is preferred because iron is an essential nutrient for virtually all micro-organisms, and sequestration of iron may most beneficially limit the growth of micro-organisms.

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In a particularly preferred embodiment, the invention provides a fluid container wherein said metal-ion sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III). It is preferred that the inorganic nanoparticles have an average particle size of less than 100 nm. It is preferred that said metal-ion sequestrant is attached to the nanoparticle by reacting the nanoparticle with a silicon alkoxide intermediate of the sequestrant having the general formula:



wherein x is an integer from 1 to 3;

R is an alkyl group; and

R' is an organic group containing an alpha amino carboxylate, a hydroxamate, or a catechol. Derivatized nanoparticles useful for practice of the invention are described in detail in U.S. patent application Ser. No. 10/822,940 filed Apr. 13, 2004 entitled DERIVATIZED NANOPARTICLE COMPRISING METAL-ION SEQUESTRANT by Joseph F. Bringley.

In a preferred embodiment the metal-ion sequestering agent is immobilized in a polymeric layer, and the polymeric layer contacts the fluid contained therein. The metal-ion sequestrant may be formed integrally within the materials comprising the bottle or may be contained within a polymeric layer directly in contact with the beverage or liquid nutrient. It is preferred that the polymer is permeable to water. It is preferred that the metal-ion sequestering agent comprises are 0.1 to 50.0% by weight of the polymer. Polymers useful for practice of the invention are described in detail in U.S. patent application Ser. No. 10/823,453 filed Apr. 13, 2004 entitled ARTICLE FOR INHIBITING MICROBIAL GROWTH by Joseph F. Bringley et al.

In a preferred embodiment, the metal-ion sequestering agent comprises an alpha amino carboxylate, a hydroxamate, or a catechol functional group. Metal-ion sequestrants suitable for practice of the invention include ethylenediaminetetraacetic acid (EDTA), ethylenediaminetetraacetic acid disodium salt, diethylenetriaminepentaacetic acid (DTPA), Hydroxylpropylenediaminetetraacetic acid (DPTA), nitrilotriacetic acid, triethylenetetraaminehexaacetic acid, N,N'-bis(o-hydroxybenzyl) ethylenediamine-N,N'-diacetic acid, and ethylenebis-N,N'-(2-o-hydroxyphenyl) glycine, acetohydroxamic acid, and desferroxamine B (the iron chelating drug desferal), catechol, disulfocatechol, dimethyl-2,3-dihydroxybenzamide, mesitylene catecholamide (MECAM) and derivatives thereof, 1,8-dihydroxynaphthalene-3,6-sulfonic acid, and 2,3-dihydroxynaphthalene-6-sulfonic acid, and siderophores molecules naturally synthesized by micro-organisms which have a very high affinity for Fe. Metal-ion sequestering agents suitable for use in the invention are described at length in U.S. patent application Ser. No. 10/822,940 filed Apr. 13, 2004 entitled DERIVATIZED NANOPARTICLE COMPRISING METAL-ION SEQUESTRANT by Joseph F. Bringley et al.

The antimicrobial active material of antimicrobial agent may be selected from a wide range of known antibiotics and antimicrobials. An antimicrobial material may comprise an antimicrobial ion, molecule and/or compound, metal ion exchange materials exchanged or loaded with antimicrobial ions, molecules and/or compounds, ion exchange polymers and/or ion exchange latexes, exchanged or loaded with antimicrobial ions, molecules and/or compounds. Suitable



materials are discussed in "Active Packaging of Food Applications" A. L. Brody, E. R. Strupinsky and L. R. Kline, Technomic Publishing Company, Inc. Pennsylvania (2001). Examples of antimicrobial agents suitable for practice of the invention include benzoic acid, sorbic acid, nisin, thymol, allicin, peroxides, imazalil, triclosan, benomyl, metal-ion release agents, metal colloids, anhydrides, and organic quaternary ammonium salts. Preferred antimicrobial reagents are metal ion exchange reagents such as silver sodium zirconium phosphate, silver zeolite, or silver ion exchange resin which are commercially available. The antimicrobial agent may be provided in a layer 15 having a thickness "y" of between 0.1 microns and 100 microns, preferably in the range of 1.0 and 25 microns.

In another preferred embodiment, the antimicrobial agent comprising a composition of matter comprising an immobilized metal-ion sequestrant/antimicrobial comprising a metal-ion sequestrant that has a high stability constant for a target metal ion and that has attached thereto an antimicrobial metal-ion, wherein the stability constant of the metal-ion sequestrant for the antimicrobial metal-ion is less than the stability constant of the metal-ion sequestrant for the target metal-ion. These are explained in detail in U.S. patent application Ser. No. 10/868,626 filed Jun. 15, 2004 entitled AN IRON SEQUESTERING ANTIMICROBIAL COMPOSITION by Joseph F. Bringley et al.

In a preferred embodiment, the antimicrobial agent comprising a metal ion exchange material, is exchanged with at least one antimicrobial metal ion selected from silver, copper, gold, nickel, tin or zinc.

Referring to FIG. 3, there is illustrated an embodiment of a fluid container 5 made in accordance with the present invention. The container 5, which in the embodiment illustrated is a bottle, is made of a material that comprises a barrier layer 22, an outer polymeric layer 20 and an inner polymeric layer 40 between said barrier layer 22 and outer polymeric layer 20. The inner polymeric layer 22 contains a metal-ion sequestrant 35. The barrier layer 22 preferably does not contain the metal-ion sequestrant 35. The outer layer 20 may provide several functions including improving the physical strength and toughness of the article and resistance to scratching, marring, cracking, etc. However, the primary purpose of the barrier layer 22 is to provide a barrier through which micro-organisms 25 present in the contained fluid cannot pass. It is important to limit or eliminate, in certain applications, the direct contact of micro-organisms 25 with the metal-ion sequestrant 35 or the layer containing the metal-ion sequestrant 35, since many micro-organisms 25, under conditions of iron deficiency, may bio-synthesize molecules which are strong chelators for iron and other metals. These bio-synthetic molecules are called "siderophores" and their primary purpose is to procure iron for the micro-organisms 25. Thus, if the micro-organisms 25 are allowed to directly contact the metal-ion sequestrant 35, they may find a rich source of iron there and begin to colonize directly at these surfaces. The siderophores produced by the micro-organisms may compete with the metal-ion sequestrant for the iron (or other bio-essential metal) at their surfaces. However the energy required for the organisms to adapt their metabolism to synthesize these siderophores will impact significantly their growth rate. Thus, one object of the invention is to lower growth rate of organisms in the contained liquid. Since the barrier layer 22 of the invention does not contain the metal-ion sequestrant 35, and because micro-organisms are large, the micro-organisms may not pass or diffuse through the barrier layer 22. The barrier layer 22 thus prevents contact of the micro-

organisms with the polymeric layer 40 containing the metal-ion sequestrant 35 of the invention. It is preferred that the barrier layer 22 is permeable to water. It is preferred that the barrier layer 22 has a thickness "x" in the range of 0.1 microns to 10.0 microns. It is preferred that microbes are unable to penetrate, to diffuse or pass through the barrier layer 22. Sequestrant 35 with a sequestered metal ion is indicated by numeral 35'.

Still referring again to FIG. 3, the enlarged sectioned view of the fluid container 5 shown in 3, illustrates a bottle having barrier layer 22, which is in direct contact with the liquid nutrient 10, an inner polymeric layer 40 and an outer polymeric layer 20. However, the bottle of FIG. 2 comprises an inner polymeric layer 15 that does not contain any metal-ion sequestering agents. In the prior art bottle illustrated in FIG. 2, the micro-organisms 25 are free to gather the "free" iron ions 30. In the example shown in FIG. 3, the inner polymer 40 contains an immobilized metal-ion sequestering agent 35 such as EDTA. In order for the metal-ion sequestering agent 35 to work properly, the inner polymer 40 containing the metal-ion sequestering agent 35 must be permeable to aqueous media. Preferred polymers for layers 22 and 40 of the invention are polyvinyl alcohol, cellophane, water-based polyurethanes, polyester, nylon, high nitrile resins, polyethylene-polyvinyl alcohol copolymer, polystyrene, ethyl cellulose, cellulose acetate, cellulose nitrate, aqueous latexes, polyacrylic acid, polystyrene sulfonate, polyamide, polymethacrylate, polyethylene terephthalate, polystyrene, polyethylene, polypropylene or polyacrylonitrile. A water permeable polymer permits water to move freely through the polymer 40 allowing the "free" iron ion 30 to reach and be captured by the agent 35. An additional barrier 22 may be used to prevent the micro-organism 25 from reaching the inner polymer material 40 containing the metal-ion sequestering agent 35. Like the inner polymer material 40, the barrier layer 22 must be made of a water-permeable polymer as previously described. The micro-organism 25 is too large to pass through the barrier 22 or the polymer 40 so it cannot reach the sequestered iron ion 30 now held by the metal-ion sequestering agent 35. By using the metal-ion sequestering agents 35 to significantly reduce the amount of "free" iron ions 30 in the liquid nutrient 10, the growth of the micro-organism 25 is eliminated or severely reduced.

In the embodiment shown in FIGS. 4, 5, and 6, the metal-ion sequestering agent 35 is contained in the bottle cap 50 instead of on the inside surface of the bottle 5. An inner portion 45 of the cap 50, which is in intimate contact with the liquid nutrient 10, is made of a hydrophilic polymer 55 containing the metal-ion sequestering agent 35 such as EDTA as described above. In some situations, the bottle may need to be placed in the inverted position in order for the sequestrant to become in contact with the contained nutrient. The cap 50 may also have the barrier layer 22 to further prevent the micro-organisms 25 from reaching the sequestered "free" iron 30. In another embodiment (not shown) the cap sealing material could be an open cell foamed structure whose cell walls are coated with the sequestering material.

In still another embodiment, the sequestering agent 35 may be in a hydrophilic polymeric insert 52 that is placed in the bottle 5 as illustrated in FIG. 4. The insert 52 may be instead of or in addition to the sequestrant in the cap 50 or interior of the bottle. The insert 52 is placed in the bottle 5 but unfolds making it too large to exit the bottle 5. In another version, the insert 52 is molded into the bottom of the bottle 5.



Referring to FIGS. 7 and 8, there is illustrated another modified embodiment of a container made in accordance with the present invention, like parts indicating like parts and operation as previously described. In this embodiment the metal-ion sequestering agent 35 is contained in a projecting member 60 that extends from cap 50 into the bottle 5 so that it will be in intimate contact with the liquid nutrient 10. In the embodiment, the projecting member is in the configuration of a straw that can later be used to drink the liquid content in the bottle. Like the hydrophilic polymer material lining of the inside of the bottle 5 and bottle cap 50, the extension 60 or straw is made of a hydrophilic polymer 65 containing the metal-ion sequestering agents 35 such as EDTA as described in FIG. 3. When the bottle 5 is filled with the liquid nutrient 10 such as an isotonic, and is capped, the straw 60 protrudes from the cap 50 into the solution 10 allowing the "free" iron ions 35 to be sequestered from the liquid nutrient 10. The straw 60 may also have the barrier layer 22 to further prevent the micro-organisms 25 from reaching the sequestered "free" iron ions 30. The outer layer 20 may also be made of a material similar to barrier layer 22 so that "free" iron ions 30 can reach the sequestrant 35 from the outside of the straw 60.

In the example shown the extension is a straw but the extension can be of any shape just as long as it extends into the food or beverage establishing intimate contact.

Referring to FIGS. 9 and 10, there is illustrated another embodiment of a bottle 5 made in accordance with the present invention. In this embodiment, the metal-ion sequestering agent 35 is applied to the inside surface 80 of the bottle 5 by spraying a metal-ion sequestering agent 35, for example EDTA, on to the inside surface of the bottle through a supply tube 85 using a spherical shaped nozzle assembly 90. The nozzle assembly 90 is moved up and down in the direction of the arrow 95 while the metal-ion sequestering agent 35 is sprayed as indicated by the arrows 100. The method of applying coatings to glass, metal or plastic containers is well known to those skilled in the art. FIG. 10 illustrates an enlarged partial cross sectional view of the portion of the bottle of FIG. 9 where the spray coating 105 of the ion sequestering agent 35 has been applied. As previously discussed in FIG. 3, like numerals indicate like parts and operations. It is of course understood that the inner layer containing the sequestrant may be applied or formed on the inside surface of the container in any appropriate manner. The bottle 5 in this embodiment may be made of any appropriate plastic or glass material.

Referring to FIGS. 11 and 12, there is illustrated yet another modified container 110 made in accordance with the present invention. In particular the container comprises juice/drink box 110 for containing a liquid beverage. The box 110 is made of a sheet material that comprises inner layer 115, a middle layer 120 made of a hydrophobic polymer material, and an outer layer 125. The inner layer 115 is in direct contact with the liquid nutrient 10 and is made of a hydrophilic polymer containing the metal-ion sequestering agent 35 such as EDTA as described above in FIG. 3. As previously discussed in FIG. 3, like numerals indicate like parts and operations. The outer layer 125 may comprise a foil wrap.

Referring to FIGS. 13 and 14, there is illustrated yet another modified embodiment of a container 130 made in accordance with the present invention. In the embodiment, the container comprises a stand up pouch 130. The pouch 130 comprises an inner layer 135 made of a hydrophilic polymer material, and an outer layer 140. The outer layer 140 may be made of a polymer such as Mylar™ with a

metalized coating 145. The inner layer 135 is in direct contact with the liquid nutrient 10 and is made of a hydrophilic polymer containing the metal-ion sequestering agent 35 such as EDTA as described above in FIG. 3. The stand up pouch 130 may also have the barrier layer 22 not shown to further prevent the micro-organisms 25 from reaching the sequestered "free" iron 30. As previously discussed in FIG. 3, like numerals indicate like parts and operations.

Referring to FIGS. 15 and 16, there is illustrated still another modified container made in accordance with the present invention. In this embodiment the container comprises a bag 150. The bag 150, which is intended to hold an aqueous material, comprises an inner layer 155 made of a hydrophobic polymer material and an outer layer 160. The outer layer 140 may be made of a polymer such as polyethylene terephthalate. The inner layer 155 is in direct contact with the aqueous material 155 and is made of a hydrophilic polymer containing the metal-ion sequestering agent 35 such as EDTA as described above in FIG. 3. The bag 150 may also have the barrier layer 22 not shown to further prevent the micro-organisms 25 from reaching the sequestered "free" iron 30. As previously discussed in FIG. 3, like numerals indicate like parts and operations.

The juice box 110, the pouch 130 and the bag 150 may be constructed from a base web 170 as illustrated in FIG. 17. After the base web 170 is formed, the hydrophilic layer 175 is applied via a coating assembly 180 comprised of a reservoir 185, an applicator 190 and a drive mechanism (not shown) to form the hydrophilic inner layer 175 containing the metal-ion sequestering agent 35 as described above in FIG. 3. Other methods of forming and of making webs and applying a coating such as coextrusion maybe used. It is of course understood that any suitable technique or process may be used for applying a coating on supporting web as long as the coating has the appropriate sequestrant.

Referring to FIGS. 18 and 19 there is illustrated a modified container 220 made in accordance with the present invention. In this embodiment, the container 220 comprises a can. The can 200 is made of a metal material such as aluminum or steel, and has a top and a bottom, which may or may not be made as separate piece. The can 200 may also have a lining 205, which is in direct contact with the aqueous material 155 and intended to prevent corrosion of the metal by the contents of the can. The construction of metal cans is well known by one skilled in the art. The lining 205 may include a hydrophilic polymer containing the metal-ion sequestering agent 35 or have a hydrophilic polymer strip 210 containing metal-ion sequestering agent 35 made as part of lining 205 of the can 200. The strip 210 may have a width "w" of between 1 millimeter and 30 millimeters and be spaced at intervals around the inside circumference of the can 200 and a depth "d" of -1.0 to 10 micrometers. In still another embodiment, the sequestering agent 35 may be in a hydrophilic polymeric insert 52. The insert 52 is placed in the can 200 but unfolds making it too large to exit the can 200. The insert 52 may be simply placed on the bottom of the container or if desired secured to the interior surface of the container in some fashion. The metal-ion sequestering agent performs as previously described above in FIG. 3.

Referring to FIG. 20, there is illustrated a cross-sectional view of a filter assembly 220 comprising an inlet port 225, an outlet port 230, and a filter 235. The filter 235 contains an immobilized metal-ion sequestering agent as previously described. As the solution flows through the filter assembly 220 in the direction indicated by the arrows 240, and through the filter 235, the metal ions in the solution are sequestered and removed by the metal-ion sequestering agent 245.



In a preferred embodiment using a filter assembly the solution would be continuously circulated through the filter assembly for a period ranging from 30 minutes to 24 hours until the metal ion co-concentration is reduced to a level to minimize or eliminate microbial growth as described in U.S. patent application Ser. No. 10/822,940 filed Apr. 13, 2004 entitled DERIVATIZED NANOPARTICLE COMPRISING METAL-ION SEQUESTRANT by Joseph F. Bringley et al.

An example of removal is shown in the graph illustrated in FIG. 25.

Referring to FIG. 21, there is illustrated a cross sectional view of a fluid bed ion exchange assembly 250 comprising a holding tank 255, an inlet port 260, an outlet port 265, and a fluid bed 270 containing a metal-ion sequestering material 275 made in accordance with the present invention. The solution 280 flows into the fluid bed ion exchange assembly 250 via inlet port 260 as indicated by arrow 285 through the metal-ion sequestering material 275 in fluid bed 270 as indicated by arrows 290 and out the outlet port as indicated by arrow 295.

FIG. 22 is an enlarged partial view of a portion of the fluid bed 270 containing a metal-ion sequestering material 275. An example of the metal-ion sequestering material 275 comprises a core material 300 and a shell material 305 made of the metal-ion sequestering agent 35 as described in U.S. patent application Ser. No. 10/822,940 filed Apr. 13, 2004 entitled DERIVATIZED NANOPARTICLE COMPRISING METAL-ION SEQUESTRANT by Joseph F. Bringley et al. As previously described above in FIG. 21, the solution 280 containing "free" metal ions 310 flows through the fluid bed 270 as indicated by the arrows 315. As the solution 280 flows through the fluid bed 270 the shell material 305 made of the metal-ion sequestering agent 35 gathers the metal ions 320 removing them from the solution, which then flow out through the outlet port 265.

In a preferred embodiment the flow rate through the bed will be such that the solution is in contact with the bed for 1 to 30 minutes during which the metal ion concentration will be reduced by at least 80% and microbial growth will be minimized and/or eliminated.

For example removal by particles in a simulated fluid bed assembly is shown in the graph illustrated in FIG. 26.

While in the embodiments discussed, the iron sequestering agent or antimicrobial agent may be provided only on a portion of the contacting surface of the bottle or other container. For example, but not limited to, the agents may be provided only on the body portion of a bottle and not the neck portion.

While in many of the embodiments illustrated a barrier layer is not discussed, it is to be understood that a barrier layer 22 may be provided in any of the embodiments for preventing the microbes (micro-organism) from contacting the sequestrant.

## EXAMPLES AND COMPARISON EXAMPLES

### Materials:

Colloidal dispersions of silica particles were obtained from ONDEO Nalco Chemical Company. NALCO® 1130 had a median particle size of 8 nm, a pH of 10.0, a specific gravity of 1.21 g/ml, a surface area of about 375 m<sup>2</sup>/g, and a solids content of 30 weight percent. N-(trimethoxysilylpropyl ethylenediamine triacetic acid, trisodium salt was purchased from Gelest Inc., 45% by weight in water.

Preparation of derivatized nanoparticles. To 600.00 g of silica NALCO® 1130 (30% solids) was added 400.00 g of

distilled water and the contents mixed thoroughly using a mechanical mixer. To this suspension, was added 49.4 g of N-(trimethoxysilyl) propylethylenediamine triacetic acid, trisodium salt in 49.4 g distilled water with constant stirring at a rate of 5.00 ml/min. At the end of the addition the pH was adjusted to 7.1 with the slow addition of 13.8 g of concentrated nitric acid, and the contents stirred for an hour at room temperature. Particle size analysis indicated an average particle size of 15 nm. The percent solids of the final dispersion was 18.0%.

Preparation of the immobilized metal-ion sequestrant/antimicrobial: 200.0 g of the above derivatized nanoparticles were washed with distilled water via dialysis using a 6,000-8,000 molecular weight cutoff filter. The final ionic strength of the solution was less than 0.1 millisemens. To the washed suspension was then added with stirring 4.54 ml of 1.5 M AgNO<sub>3</sub> solution, to form the immobilized metal-ion sequestrant/antimicrobial.

### Preparation of Polymeric Layers of Immobilized Metal-Ion Sequestrants and Sequestrant/antimicrobials.

Coating 1 (comparison). A coating solution was prepared as follows: 8.8 g of a 40% solution of the polyurethane Permax 220 (Noveon Chemicals) was combined with to 90.2 grams of pure distilled water and 1.0 g of a 10% solution of the surfactant OLIN 10G was added as a coating aid. The mixture was then stirred and blade-coated onto a polymeric support using a 150 micron doctor blade. The coating was then dried at 40-50° C., to produce a film having 5.4 g/m<sup>2</sup> of polyurethane.

Coating 2. A coating solution was prepared as follows: 171.2 grams of the derivatized nanoparticles prepared as described above were combined with 64.8 grams of pure distilled water and 62.5 g of a 40% solution of the polyurethane Permax 220 (Noveon Chemicals). 1.5 g of a 10% solution of the surfactant OLIN 10G was added as a coating aid. The mixture was then stirred and blade-coated onto a polymeric support using a 150 micron doctor blade. The coating was then dried at 40-50° C., to produce a film having 5.4 g/m<sup>2</sup> of the derivatized nanoparticles and 5.4 g/m<sup>2</sup> of polyurethane.

Coating 3. A coating solution was prepared as follows: 171.2 grams of the derivatized nanoparticles prepared as described above were combined with 33.5 grams of pure distilled water and 93.8 g of a 40% solution of the polyurethane Permax 220 (Noveon Chemicals). 1.5 g of a 10% solution of the surfactant OLIN 10G was added as a coating aid. The mixture was then stirred and blade-coated onto a polymeric support using a 150 micron doctor blade. The coating was then dried at 40-50° C., to produce a film having 5.4 g/m<sup>2</sup> of the derivatized nanoparticles and 8.1 g/m<sup>2</sup> of polyurethane.

Coating 4. A coating solution was prepared as follows: 138.9 grams of the derivatized nanoparticles prepared as described above were combined with 97.1 grams of pure distilled water and 62.5 g of a 40% solution of the polyurethane Permax 220 (Noveon Chemicals). 1.5 g of a 10% solution of the surfactant OLIN 10G was added as a coating aid. The mixture was then stirred and blade-coated onto a polymeric support using a 150 micron doctor blade. The coating was then dried at 40-50° C., to produce a film having 4.4 g/m<sup>2</sup> of the derivatized nanoparticles and 5.4 g/m<sup>2</sup> of polyurethane.

Coating 5. A coating solution was prepared as follows: 12.8 grams of the immobilized metal-ion sequestrant/antimicrobial suspension prepared as described above was combined with to 77.4 grams of pure distilled water and 8.8 g of



a 40% solution of the polyurethane Permax 220 (Noveon Chemicals). 1.0 g of a 10% solution of the surfactant OLIN 10G was added as a coating aid. The mixture was then stirred and blade-coated onto a polymeric support using a 150 micron doctor blade. The coating was then dried at 40-50° C., to produce a film having 2.7 g/m<sup>2</sup> of the immobilized metal-ion sequestrant/antimicrobial, 0.06 g/m<sup>2</sup> silver-ion and 5.4 g/m of polyurethane.

Now referring to FIGS. 23 and 24, there is illustrated another embodiment of the web 170 shown in FIG. 17 used for the manufacture of the box 110, pouch 130 or bag 150 made in accordance with the present invention. In the embodiment shown an electro-photographic device employs magnetic brush technology to apply toner particles 400 comprised of the metal-ion sequestering agents 35 in a predetermined pattern 475 to the web 170 forming a metal-ion sequestering web 410. The predetermined pattern may comprise the entire surface of said web 170 or selected locations on said web 170. In the example shown the electro-photographic device uses the web 170, which is driven in the direction indicated by the arrow 405 by drive roller assembly 490. The web 170 is charged with a high voltage corona via a charger 420 and selectively discharged with light from a light source 430 such as an LED array to form an electrostatic pattern 440. The electrostatic pattern is developed with the negatively charged toner particles by a magnetic brush 460 composed of the negatively charged toner particles and magnetic carrier particles (not shown) on a magnetic brush 460. The charged toner particles 475 are then fused to the web 170 by heated rollers 490 to form a fused pattern 480 as the web 170 moves in the direction of the arrow 405. By laying down a pattern the edges 500 shown in FIG. 23 may be left clean to facilitate sealing of the box 110, pouch 130 or bag 150. As in the case of an electro-photographic printer the amount or density of the toner particles transferred to the web can be controlled by the amount of charge placed on the web. Using this technique, which is understood by those skilled in the art, the amount or density of metal-ion sequestering agents 35 in the form of toner particles can likewise be controlled. This allows for providing the metal-ion sequestering agent at a designated location and at a desired density on web 170. Thus, the web may be designed to meet the various needs of the user. For example, if more than one designated metal-ion is to be treated, more than one type metal-ion sequestering agent can be applied to web. In addition the density may be varied to adjust to the amount to which the designated metal ion is present in the liquid nutrient allowing for efficient use of both metal ion sequestering agents. In addition to providing different type metal-ion sequestering agents other type agents may be applied in combination, for example, the electro-photographic device may employ magnetic brush technology to apply toner particles 400 comprised of a metal-ion sequestering agent and an antimicrobial agent, as previously described, in a pattern 475 on to the web 170. The web 170 may be made of any suitable material and of any suitable construction. For example but not by way of limitation, web 170 may be made of nylon, polyester, natural or synthetic fabric material, plastic, and may be continuous, or of a fabric type weave of one or more material fibers. It is important that the material allow for the adhering of the metal-ion sequestering agent and allow appropriate interaction with the liquid agent.

#### TESTING METHODOLOGY

A test similar to ASTM E 2108-01 was conducted where a piece of a coating of known surface area was contacted with a solution inoculated with micro-organisms. In particular a piece of coating 1×1 cm was dipped in 2 ml of growth

medium (Trypcase Soy Agar 1/10), inoculated with 2000CFU of *Candida albicans* (ATCC-1023) per ml. Special attention was made to all reagents to avoid iron contamination with the final solution having an iron concentration of 80 ppb before contact with the coating.

Micro-organism numbers in the solution were measured daily by the standard heterotrophic plate count method.

The bar graph shown in FIG. 27 demonstrates the effectiveness of the inventive examples. The yeast population which was exposed to the comparison coating 1 (which contained no derivatized nanoparticles) showed a growth factor of one thousand during 48 hours (a 1000-fold increase in population). The yeast population which was exposed to the example coatings 2-4 (containing derivatized nanoparticles) showed growth factors of only 1-4. This is indicative of a fungostatic or bio-static effect in which the population of organisms is kept at a constant or near constant level, even in the presence of a medium containing adequate nutrient level. The yeast population which was exposed to the example coating 5 (derivatized nanoparticles that had been ion exchanged with silver ion—a known antimicrobial) showed a fungicidal effect (the yeast were completely eliminated). The low level of silver when coated by itself without the nanoparticles would not be expected to exhibit this complete fungicidal effect, and there appears to be a synergistic effect between the iron sequestration and the release of antimicrobial silver.

As can be seen from the bar graph illustrated in FIG. 27, significant improved results may be obtained when a metal-ion sequestering agent is used in conjunction with an antimicrobial agent. The combined agents reduced the level of microbes to lower level than when first introduced and then maintained the reduced level of microbes in the liquid nutrient.

The invention has been described in detail with particular reference to certain preferred embodiments thereof but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

#### PARTS LIST

5 fluid container/bottle  
 10 liquid nutrient  
 15 inner polymeric layer  
 20 outer polymeric layer  
 25 barrier layer  
 25 micro-organism  
 30 "free" iron ion  
 35 metal-ion sequestering agents  
 35' metal-ion sequestering agent with a sequestered metal ion  
 40 hydrophilic polymer  
 45 inner portion  
 50 bottle cap  
 52 insert  
 55 hydrophilic polymer  
 60 extension (straw)  
 65 hydrophilic polymer  
 80 inside surface  
 85 supply tube  
 90 spherical shaped nozzle assembly  
 95 arrow  
 100 arrow  
 105 spray coating  
 110 juice box  
 115 inner layer  
 120 middle layer



**125** outer layer  
**130** pouch  
**135** inner layer  
**140** outer layer  
**145** coating  
**150** bag  
**155** aqueous material  
**160** inner layer  
**165** outer layer  
**170** base web  
**175** hydrophilic layer  
**180** coating assembly  
**185** reservoir  
**190** applicator  
**200** can  
**205** lining  
**210** strip  
**220** filter assembly  
**225** inlet port  
**230** outlet port  
**235** filter  
**240** arrow  
**250** fluid bed ion exchange assembly  
**255** holding tank  
**260** inlet port  
**265** outlet port  
**270** fluid bed  
**275** sequestering material  
**280** solution  
**285** arrow  
**290** arrow  
**295** arrow  
**300** core material  
**305** shell material  
**310** "free" metal ions  
**315** arrows  
**320** gathered metal ions

What is claimed is:

**1.** A method for inhibiting growth of microbes in a liquid having a pH equal to or greater than about 2.5, comprising the steps of:

- a. providing a filter assembly for filtering said liquid, said filter assembly having a filter having a metal-ion sequestering agent for removing designated metal ions from said liquid, said filter assembly having an inlet and an outlet for allowing said liquid to enter and leave said filter assembly, wherein the filter comprises a barrier layer and a polymeric layer, the polymeric layer contains the sequestering agent and is disposed between a surface of the filter and the barrier layer, and wherein the barrier layer is permeable to water so that the water contacts the sequestering agent but microbes cannot pass or diffuse through the barrier layer; and
- b. causing said liquid to enter said filter assembly through said inlet pass through said filter and out said outlet for a sufficient time so as to substantially reduce the amount of the designated metal ion from said liquid, wherein the growth of said microbes is inhibited.

**2.** The method of claim **1** wherein said sufficient time comprises from about 1 to 30 minutes.

**3.** The method of claim **1** wherein a concentration of said designated metal ions is reduced by at least 80%.

**4.** The method of claim **1** wherein said metal-ion sequestering agent has a stability constant greater than  $10^{10}$  with iron (III).

**5.** The method of claim **1** wherein said sequestering agent has a high-affinity for biologically important metal ions including Mn, Mg, Zn, Cu and Fe.

**6.** The method of claim **1** wherein said sequestering agent has a high-selectivity for biologically important metal ions including Mn, Mg, Zn, Cu and Fe.

**7.** The method of claim **1** wherein said sequestering agent has a high-selectivity for certain metal ions but a low-affinity for at least one other ion.

**8.** The method of claim **7** wherein said certain metal ions comprises Mn, Mg, Zn, Cu and Fe and said at least one other ion comprises calcium.

**9.** The method of claim **1** wherein said metal-ion sequestering agent has a stability constant greater than  $10^{20}$  with iron (III).

**10.** The method of claim **1** wherein said metal-ion sequestering agent has a stability constant greater than  $10^{30}$  with iron (III).

**11.** The method of claim **1** wherein said metal-ion sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached metal-ion sequestrant, wherein said inorganic nanoparticles have an average particle size of less than 200 nm and the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

**12.** The method of claim **11** wherein said inorganic nanoparticles have an average particle size of less than 100 nm.

**13.** The method of claim **11** wherein said metal-ion sequestrant is attached to the nanoparticle by reacting the nanoparticle with a silicon alkoxide intermediate of the sequestrant having the general formula:



wherein x is an integer from 1 to 3;

R is an alkyl group; and

R' is an organic group containing an alpha amino carboxylate, a hydroxamate, or a catechol.

**14.** The method of claim **1** wherein said metal-ion sequestering agent is immobilized in the polymeric layer, and the polymeric layer contacts the liquid contained therein.

**15.** The method of claim **14** wherein the polymeric layer is permeable to water.

**16.** The method of claim **14** wherein the metal-ion sequestering agent comprises about 0.1 to about 50.0% by weight of the polymeric layer.

**17.** The method of claim **1** wherein said metal-ion sequestering agent comprises an alpha amino carboxylate, a hydroxamate, or a catechol functional group.

**18.** The method of claim **1** wherein said metal-ion sequestering agent comprises a naturally synthesized siderophore molecule.

**19.** The method of claim **1** wherein the barrier layer has a thickness in the range of about 0.1 microns to about 10.0 microns.

**20.** The method of claim **1** where said liquid comprises a beverage.

**21.** The method of claim **1** wherein said filter further includes an antimicrobial agent for reducing and/or maintaining an amount of microbes in said liquid to a prescribed condition.

**22.** A method for inhibiting growth of microbes in a liquid having a pH equal to or greater than about 2.5, comprising the steps of:

- a. providing a filter assembly for filtering said liquid, said filter assembly having a filter having a metal-ion sequestering agent for removing designated metal ions



from said liquid, said filter assembly having an inlet and an outlet for allowing said liquid to enter and leave said filter assembly; and

- b. causing said liquid to enter said filter assembly through said inlet pass through said filter and out said outlet for a sufficient time so as to substantially reduce the amount of the designated metal ion from said liquid, wherein the growth of microbes is inhibited, said metal-ion sequestering agent comprises derivatized nanoparticles comprising inorganic nanoparticles having an attached metal-ion sequestrant, said inorganic nanoparticles have an average particle size of less than 200 nm, and wherein the derivatized nanoparticles have a stability constant greater than  $10^{10}$  with iron (III).

23. The method of claim 22 wherein said sufficient time comprises from about 1 to 30 minutes.

24. The method of claim 22 wherein a concentration of said designated metal ions is reduced by at least 80%.

25. The method of claim 22 wherein said metal-ion sequestering agent is immobilized on a surface of said filter and has a stability constant greater than  $10^{10}$  with iron (III).

26. The method of claim 22 wherein said sequestering agent is immobilized on a surface of said filter and has a high-affinity for biologically important metal ions including Mn, Mg, Zn, Cu and Fe.

27. The method of claim 22 wherein said sequestering agent is immobilized on a surface of said filter and has a high-selectivity for biologically important metal ions including Mn, Mg, Zn, Cu and Fe.

28. The method of claim 22 wherein said sequestering agent has a high-selectivity for certain metal ions but a low-affinity for at least one other ion.

29. The method of claim 28 wherein said certain metal ions comprises Mn, Mg, Zn, Cu and Fe and said at least one other ion comprises calcium.

30. The method of claim 22 wherein said metal-ion sequestering agent is immobilized on a surface of said filter and has a stability constant greater than  $10^{20}$  with iron (III).

31. The method of claim 22 wherein said metal-ion sequestering agent is immobilized on a surface of said filter and has a stability constant greater than  $10^{30}$  with iron (III).

32. The method of claim 22 wherein said metal-ion sequestering agent is immobilized in a polymeric layer, and the polymeric layer contacts the liquid contained therein.

33. The method of claim 32 wherein the polymeric layer is permeable to water.

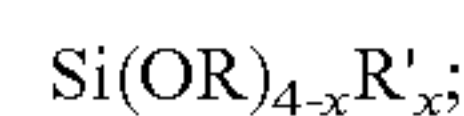
34. The method of claim 32 wherein the metal-ion sequestering agent comprises about 0.1 to about 50.0% by weight of the polymeric layer.

35. The method of claim 22 wherein said inorganic nanoparticles have an average particle size of less than 100 nm.

36. The method of claim 22 wherein said metal-ion sequestering agent comprises an alpha amino carboxylate, a hydroxamate, or a catechol functional group.

37. The method of claim 22 wherein said metal-ion sequestering agent comprises a naturally synthesized siderophore molecule.

38. The method of claim 22 wherein said metal-ion sequestrant is attached to the nanoparticle by reacting the nanoparticle with a silicon alkoxide intermediate of the sequestrant having the general formula:



wherein x is an integer from 1 to 3;

R is an alkyl group; and

R' is an organic group containing an alpha amino carboxylate, a hydroxamate, or a catechol.

39. The method of claim 22 further comprising a barrier layer and a polymeric layer, wherein the polymeric layer is between a surface of the filter and the barrier layer and wherein the barrier layer does not contain the derivatized nanoparticles.

40. The method of claim 39 wherein the barrier layer is permeable to water.

41. The method of claim 39 wherein the barrier layer has a thickness in the range of about 0.1 microns to about 10.0 microns.

42. The method of claim 39 wherein microbes cannot pass or diffuse through the barrier layer.

43. The method of claim 22 where said liquid comprises a beverage.

44. The method of claim 22 wherein said filter further includes an antimicrobial agent for reducing and/or maintaining an amount of microbes in said liquid to a prescribed condition.

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